WHEAT RESEARCH IDEATION SESSION

FINAL REPORT ON

THE PROCEEDINGS OF THE MEETING CONVENED BY:

GRAIN INSPECTION, PACKERS AND STOCKYARDS ADMINISTRATION

FEDERAL GRAIN INSPECTION SERVICE

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USDA

FGIS

WHEAT RESEARCH IDEATION SESSION

EXECUTIVE SUMMARY

For many years, import customers of North American wheat have asked for data on the functional properties of their purchase. To date, the large global wheat exporters have lacked the capability to satisfy these requests despite large research expenditures. On April 24-25, 2003, the Grain Inspection, Packers and Stockyards Administration's-Federal Grain Inspection Service (FGIS) hosted an ideation session in Kansas City, MO, to generate new avenues of research leading to rapid tests of wheat end-use functional characteristics, applicable to wheat cargo during inspection as well as other points in the value chain. FGIS invited a core group of approximately 25 leading wheat researchers, primarily from North America, to participate in the exercise. Participants were from Canadian and U.S. government research labs, private industry, non-profit organizations and universities, with expertise in the areas of protein, starch, lipids, imaging, and spectroscopy, among others.

The groups charge: To generate a prioritized list of research objectives for developing rapid market compatible tests of end use quality, such that the industry will have the rapid tests available in the marketplace by May, 2006.

The opening exercise generated a list of the most important wheat quality factors: The group brainstormed and produced a list (page 7) of more than 70 important quality factors, which when ranked for importance, yielded the top ten:

1. Gluten Strength
2. Dough Mixing Stability/Characteristics
3. Variety/Classification
4. Water Absorption
5. Biochemical Composition
6. Kernel Conformity (geometry)
7. Defects (insects, mycotoxins, pesticide, etc.)
8. Milling Yield
9. Sedimentation Volume
10. Lot Consistency

Three breakout teams discussed suitable technologies for measuring these most important quality factors (page 9). The group developed a matrix linking the most promising measurement technologies to those factors. Those technologies included:

ELISA technology
NIR, Mid-IR and Raman spectroscopy; European NIR applications
The O'Graph methods (farinograph, mixograph, alveograph)
SDS sedimentation volume
Electrophoresis
Ultrasound
Single Kernel Characterization
Imaging
Varietal Identification (as a business SOP, not as technology)

The final exercise was to brainstorm the question "How do We Develop the Promising Technology Needed to Measure the Ranked Quality Factors"?, using two breakout teams. One team discussed (page26) the following question, related to protein:

From the list of the technologies, how do we develop the most promising approaches to characterize gluten?

- NIR
- Ultrasound
- SDS
- Mid-IR
- Raman
- ELISA
- The O'Graphs

The second team was given a broader discussion question (page 38):

How do we from the list of identified technologies develop the most promising approaches to measure the following factors?

Factors	**Technologies**
Variety/Class	Imaging, SKCS, ELISA
Water Absorption	NIR, ELISA, O'Graphs
Kernel Conformity (Geometry)	not assigned
Starch Quality	not assigned
Milling Yield	SKCS, Imaging
Defects (insects, mycotoxins, pesticides)	not assigned

The protein group indicated that for short and medium term goals, technologies such as NIR, SDS, ultrasound, Mid IR, and glutomatic have promise. Longer term approaches include a combination of Mid-IR, Raman and mass spec techniques, ELISA, Chip technology, PCR and again ultrasound (page 54).

The non-protein group considered ELISA-PCR-DNA; water absorption by NIR; starch characteristics; imaging; varietal declaration and identification; defect detection; kernel conformity; as areas that might yield suitable technology within the 3 year horizon (page 57).

Efforts for finding additional research funding should be directed at CSRES and ARS national programs (by garnering industry support via congressional contact); private foundation money and other money sources (page 60).

The group should meet in 2004 to assess progress and insure the initiative stays on track, with additional researchers as necessary.

WHEAT RESEARCH IDEATION SESSION

Introduction:

Purchasers of wheat, particularly international customers, have long asked for data on the functional or end-use characteristics of their purchase. Presently, the United States official grain inspection system and the large global exporters of wheat lack the capability to provide this information, despite a large expenditure of research dollars. What has been and is needed is a rapid, reliable and cost effective means to predict functional quality parameters. Such tests, if available, could be performed during official inspection, but would also be useful to the grain industry at large for wheat segregation decisions, as well as providing breeders, producers, and exporters with valuable data that anticipate end use quality characteristics of wheat. End-use characteristics fall outside of the Grade Requirements for Wheat in the United States Standards for Grain.

On April 24-25, 2003, the Grain Inspection, Packers and Stockyards Administration's-Federal Grain Inspection Service (FGIS) hosted an ideation session in Kansas City, MO, for the purpose of generating new avenues of research leading to rapid tests of wheat end-use functional characteristics, applicable during inspection as well as other points in the value chain. FGIS invited leading wheat researchers, primarily from North America, to participate in the exercise. Participants were from Canadian and U.S. government research labs, universities, private industry, and non-profit organizations. Expertise in protein, starch, lipids, imaging, immunoassay and spectroscopy, among others, was represented.

To achieve the maximum output from the group, FGIS utilized a professional facilitator. Mr. Tim Blackburn of USDA's Animal and Plant Health Inspection Service assisted in the planning and implementation of the meeting. Mr. Blackburn has substantial experience working with high performing groups at the USDA senior managerial level. He was assisted by GIPSA personnel from the Technical Services Division. The list of participants appears at the end of this report.

To prepare for the report writing task, GIPSA made audio recordings of the meeting using mini-cassette tape recorders. The transcription process was unexpectedly long, requiring a large number of hours to complete and was due to poor audio quality mitigated by external noise; soft voices; regional speech patterns and distance between the speaker and the tape recorder. Thus some of the discussion was not decipherable. We ask that if while reading this report the reader recognizes the discussion string and can fill in missing details, they contact GIPSA such that we can add to the transcript. Also, anyone wanting to listen to the original cassettes is welcome to do, again to help decipher information.

There is value to sharing the discussion on the tapes with all interested parties. What is included is as close to verbatim as possible.

The meeting syllabus is given below:

Plenary session: Dave Shipman: Opening comments.

Self-introduction by participants.

Dave Shipman (GIPSA-FGIS): assign the task to the group.

Steve Tanner (GIPSA-FGIS): GIPSA Research philosophy.

John Oades (U.S. Wheat Associates): Import customer needs.

Ron Olson (General Mills, Inc.): Domestic industry considerations.

Group Brainstorming: ***Identify meaningful factors indicative of end use quality.***

Rank for top ~10 factors.

Presentations on technologies: ELISA, NIR^{+}, Ultrasound, SKCS' and Imaging.*

Group Brainstorming and Discussion: ***Identify other relevant technologies, associated research****.*

Breakout: Linking the factors to the technology (building a Matrix).
"What rapid tests can we use to measure the ranked quality factors?"

Group reports on Breakout.

Breakout: Developing the needed technologies (rapid tests).
"How do we develop the promising technologies needed to measure the ranked quality factors?" from the Matrix.

Group reports on Breakout.

Plenary session: Discussion of research funding options, including GIPSA funding, extramural grants, etc.

**ELISA: Enzyme Linked ImmunoSorbent Assay*
$^{+}$ NIR: Near InfraRed (spectroscopy)
' SKCS: Single Kernel Characterization System

David Shipman's remarks, which set the tone for the meeting, are excerpted here:

> "…During my 26-year career with USDA, I have attended a number of meetings that focus on grain quality. The results from those meetings have been mixed if you were to poll the participants.
>
> The U.S. grain and oilseed markets are undergoing significant change due to technological advances (breeding, production, processing, information management), global competition (especially the wheat market -- our market share has dropped dramatically) and consumer demand. The traditional push commodity market (grow it and they will buy it) has transitioned to a pull market (know your customers and produce wheat they want) where product differentiation is increasingly more important.
>
> This is especially true for wheat. Consistent functional quality in wheat is closely related to economic value. This is true to our domestic customers and increasingly important to our overseas customers. Of course "functionality" differs among and between buyers making the chore of measuring wheat end-use functionality increasingly difficult.
>
> We have a number of technologies currently available for measuring functional quality but unfortunately these technologies have not been reduced to practice such that they are market applicable in the sense of being fast, relatively simple to perform, inexpensive and highly reliable and repeatable. We need technology that can operate at country elevators and export terminals and all points in between.
>
> And we need this technology now! We need to explore multiple approaches and interim solutions if necessary. We can't wait for the elusive black box that tells us all.
>
> We have gathered a talented pool of scientists who represent areas of expertise including protein, starch and lipids, hard and soft wheat, spectroscopy, imaging, biochemistry, and more. We have Canadian and U.S. participants who have spent a lot of time with importing customers and who have heard what sort of quality information these buyers are interested in.
>
> This is not just a hard wheat meeting, nor is it an NIR meeting. It is not an ARS research meeting nor is it a strictly U.S. research meeting. The collective expertise in this room encompasses all wheat classes, multiple technologies and national borders.
>
> If we can ultimately develop the means to offer rapid tests of functional quality, these tests will be of value not only of the federal grain agencies of the US and Canada but also to the wheat industry of North America in general, as well as others. Science will continue to bring a greater diversity of wheat varieties to the marketplace; we now need science to help us differentiate those qualities so the market can deliver the right quality to the right end-user. In brief, American wheat farmers need your help.
>
> Thus is our task: to generate a prioritized list of research objectives for developing rapid market compatible tests of end use quality. I challenge this group to meet this goal by May, 2006 --3 years from now. Let's have the rapid tools available in the market."

Steve Tanner provided criteria for tests of functional quality in an inspection setting. The criteria are: Accurate; Repeatable/Precise; Inexpensive; Safe; Rapid (preferably 3 minutes or less); User friendly; Robust and Validatible/Traceable. Steve underscored the connection to the goal of differentiating grain for the marketplace and elucidating value.

Moving into the first brainstorming session, the group was tasked with coming up with as many "wheat quality factors" as could be listed. No parameters had been set regarding what constituted a "wheat quality factor", thus over 80 factors were listed. (Lively discussion occurred later on what the term "quality" should imply.) When regrouped to account for repeat and overlap, the final list totaled 73 factors. They are shown below, in no particular order:

Lipids
Contaminants
Tannin Content
Moisture
Mechanical Properties
Cookie Spread
Odor
Alpha Amylase
Starch Swelling Composition
Rate of Hydration
Bran Thickness
Protein Fingerprint
Grain Cleaning
Machinability
Test Weight
Insects
Pigments
Disease Resistance
Protein Content
1000 Kernel Weight
Non-GMO
Dough Extensibility
Heat Damage
Sedimentation Volume
Starch Quality (waxy)
Pharmaceutical Wheat
Fermentation Tolerance
PPO
Wheat Cleanliness
Calorie Content
Baking Strength
Variety/Classification
Nutritional Characteristics
Protein Secondary Structure
Security (bioterrorism)
Toxicity Through Supply Chain
Water Soluble Proteins
Flavor
Amino Acid Profile
Dough Mixing Stability/Characteristics
Ash (flour/wheat)
Falling Number
Phenotypic Stability
Kernel Conformity (geometry)
Lot Consistency
Color
Price
Mixing Strength
Waxy
Germination
Pentosans
Production Environment
Loaf Volume
Water Absorption
Gluten Thermal Stability
Enzyme Composition
Balance of Extensibility
Monomeric Protein Content
Gas Retention Power
Genetically Modified Traits
Wet Gluten
Defects (insects, mycotoxins, pesticide)
Biochemical Composition
Milling Yield
Glutenins
Swelling Index of Glutenin
Starch Damage
Final Product
Pharmaceutical Uses
Vitreousness
Kernel Texture (hardness/softness)
Gluten Strength
Ease of Processing

The group voted on which of the above factors were most important. The ranked order of the top ten quality factors is shown below:

1. Gluten Strength
2. Dough Mixing Stability/Characteristics etc.)
3. Variety/Classification
4. Water Absorption
5. Biochemical Composition
6. Kernel Conformity (geometry)
7. Defects (insects, mycotoxins, pesticide,
8. Milling Yield
9. Sedimentation Volume
10. Lot Consistency

It should be noted that Gluten Strength is a broad concept and several of the factors from the original list would fall within the range. Item 2, Dough Mixing Stability/ Characteristics may be considered by some to be one of those factors. Ranked factor 11 (not shown) was Enzyme Composition, while factors 12-16 (not shown) were primarily related to gluten strength as well. Ranked factor 17 (not shown) was Starch Quality. Having established this list of the 10 most important quality factors, the group listened to presentations on various wheat measurement technologies. The presentations were:

Barrie Kitto (ELISA): *Immunoassays for Agriculture*
Tom Pearson (SKCS): *Single Kernel Characterization Systems for Wheat Quality Measurement*
Steven Delwiche (Imaging): *Digital Imaging of Wheat Brief Overview*
Martin Scanlon (Ultrasound): *The Application of Ultrasound to Wheat Quality Evaluation*
Brad Seabourn (NIR): *Near Infrared Spectroscopy: Fundamentals and Potential for use in Wheat Marketing*

Additional technologies were discussed by various group members. These discussions succeeded at allowing participants to get some initial background on research areas they might not normally hear discussed.

Armed with a list of quality factors and having heard presentations on various technologies, the participants were divided into 3 breakout groups, established at random, to discuss the question: ***"What rapid tests can we use to measure the ranked quality factors?"*** The breakout groups were given one hour to discuss, or develop a "Matrix", of those technologies which appeared most promising, for determining or analyzing, those top ten quality factors.

The transcripts of two breakouts are given below. The third groups tape was defective and could not be transcribed. We remind the reader that the transcripts are as faithful as possible given the audio quality and other aural considerations. Participants are asked to fill in any missing material or correct any errant comment. Symbols such as ++, //, **, etc, are used to differentiate comments of numerous individuals participating in discussions. *Italics* denote editorial notes.

Breakout session with **Ron Bicsak** as moderator:

Ron: the first one we are going to talk about inside this group gluten strength, dough mixability, stability characteristics are going to be considered as one topic. So what kind of existing technologies do we have that we measure those things with now?

Ron: Alveograph, other o'graphs; I can list those.

Are there any other technologies, some of the ones we talked about in there, like straight chemical methods, or mass spec or anything like that we think we can use to determine these quality factors?

We've done some work with ?? insoluble polymers which is directly related to mixing strength but we also have a paper coming out on near IR calibrations to do insoluble polymers. This will be a direct method for mixing strength.

So NIR?

So we are not specifically naming farinograph, mixograph, etc?

Ron: I will put them down and list them all

You said insoluble polymers are directly related to mixing strength. You've got a quick test, a chemical test…

We can do 150 of them a day.

Take out the solubles, dry it run it on a Leco nitrogen analyzer. But we now have an NIR calibration for that.

Ron: the way I put it is I put NIR and you are looking at insoluble polymers.

Yes.

Is this for flour or whole grain.

This is flour, but I imagine you could do it for whole grain

With the alveograph we tried to get a correlation (or calibration) with the for ??the ?? value ?? Ukraine. So they are working on that. So it is possible to do.

The number one thing is accuracy and precision, right? So I think we will need r square or some predictability. Do we add them as we talk about them. Are we talking about point 9 or what?

…if they aren't highly correlated you are going to have a wreck

much over talking discussion is about correlation coefficients

you're looking at more the allocated…
you're ..there for segregating versus quantitative versus qualitative…guess at it.
Well he said he got point 8.
Well yeah but he's guessing. it's a quantitative …
I think there are lots of other considerations when you look at NIR calibrations. One of them is how good is the reference method. As we start looking at the o'graph, not the top of the line as far as reference methods. The other one is the information Brad submitted, that Phil put together on RPD (*the standard deviation of reference data of validation samples divided by the standard deviation of differences between NIR and reference data)*. Sometimes that's a little misleading because to get a high RPD you use a real wide range and if you are really interested in a small chunk of that, if you

have a huge range and a high having a high RPD doesn't mean anything useful. So the statistics are tough on this.

Ron: And one of the things we need to remember is what we're doing is looking at where possible, we're looking at research maybe going into this to refine or define it so that it meets the needs that we have for something that we have existing. It doesn't necessarily have to meet it today but it's a possibility and that's why we can't think of the statistics of it as it is today. We have to think of the possibility of it being able to be used in the system to replace something that…

I have to think about that Ron because there's a timeframe that Dave talked about, which is 2006. That's 2-3 years. Now we're not talking about a research project here as far as I know. The way I understand research, we are not talking about a beginning research project.

But if it's already being used in another country and the research has already been done and the refinement may not take 3-5 yrs so we shouldn't limit ourselves to something we know we can absolutely do in 3 years time. This list here is a wish list-a possibility list.

Another criteria is to not get too hung up with the accuracy and repeatability. It's a question of is it equal to or better than what we currently do. Is it better than we currently have, and that's been described as having a test that takes 3 minutes vs. a 45 minute visual segregation. That's still an enhancement to the system. So the fact is that something that has a wide tolerance is not all that tolerant. It may not a great r sq but still be a step forward.

A good point.

I'd like to make a suggestion too. NIR is going to show up in a lot of these things, but one of the things we'll also look at are other technologies so we've got 5 to talk about this morning, that made the other list of other potential technologies. So maybe we ought to go through those technologies and say "does this have an opportunity to be applied in this particular area?". And if it does, list it up there. Otherwise we'll …So would you make a list of those?
Ron: well I made a list of the methods that were listed, this last list .
Like the DNA based?
Ron: DNA, straight chemistry, ?? particle technology; I made a list of those. Were those others.
Well this morning we had NIR, ELISA, Ultrasound.
Ron: OK do we want those?
Well if you can link those, you can mention those first two as being together, so I guess that's our next task is to *?link?* those with the technology that we heard about, that we know about.
Ron: OK so we had NIR, ELISA, single kernel (SKCS), mid IR, Raman.

If we go back to quality, what is commercially measured today is protein, moisture, wet gluten, zeleny and the alveograph values, with NIR. That can be done. And you can do wet gluten pretty good with the NIR.

Are others important?

Well maybe for the export market. At US Wheat, we looked at that kind of thing and all my numbers looked way higher than the ranges. So will it make a difference for us with hard wheat?

??...some way of changing that so that we'd know that if you say, instead of having a number, you have a number greater than 635 or whatever is appropriate. Then you don't have to worry about going through the whole ??

Discussion of wet gluten and alveograph NIR calibrations in Europe

Ron: OK we need to focus back on the first two. Are there any of those technologies besides NIR that we want put down as possible?

How did wet gluten not get in our top five list? (*Editors note: wet gluten received 2 votes and was in a 7-way tie for13th place.)* Looking at this list, is there any other technology we want to list in this group under possible…

I would sure like to see a list of technology looked at, particularly for ? purposes.

Ron: Like ELISA?

No I you want to look at quick. ELISA is no way to be there so NIR works, we already have it in the right place already. Test kits are a bit away. Particularly for not wanting to look at ??

You know John, ELISA would be nice but I just don't if it would be worth it in this particular area. I don't know how you could design an ELISA that could give you some elucidation about ???

You might need another step in there. ?? protein marker to do that.

What about the ultrasound? That sounded to me this morning like a real logical application directly to the ?.

**…I guess one of the things we're doing commercially, thinking of the problems we have in trying to decide how to bin wheat and how to segregate, in my mind I keep going back to on line things and off line batch processes kinds of things and just looking at the list there I think NIR, imaging, mid-NIR and Raman. Those are the kinds of things we ought to focus on. If we can train those systems appropriately because with any of the others you are not talking about rapid; you're talking about ??

== I want to add to that. Some of the work that's been going on with trying to move the RVA technology out from the ports and having the inspectors do it. Repeatability is not something they understand or care about. Weighing is not accurate. Spilling occurs. A technique which could work very well in a laboratory isn't the same as taking a technology out to the port or back to the elevator, so I think that the end user use factor is critical. You may have to give up accuracy to some degree so then you could move other techniques back to a centralized type of setup.

Another point that is critical is that you assume the contract covered and the grade is segregated at the first point of delivery and follows through the system, not necessarily as an IP in the sense that "I'm going to identify this truckload that's going to go to this customer" I'm simply saying that as you do basic segregation for functional quality, then if that ultimately is where you have contract terms, then repeatability of that test that's in the contract is important. It's going to hook directly to money. If it isn't there, when the customer get it on the other end, then the whole system begins to come apart, like or not, it's critical.

Ron: so far we have NIR ELISA ultrasound mid IR and

I think we need mass spec too. I think that in the future anything that has to do with protein, that lumps any kind biochemicals are going to be done with mass spec. they are getting cheaper and cheaper because you get ?? characterization..

Are they miniaturizing this stuff?
Oh yes. They are getting tiny.

What about, I know nothing about it, I'm sure you know about high mobility, basically a ?? of mass spec, and less expensive, ?? at airports right now. I don't know if it would be useful in the same way?

Ron: do we want to list these things then?

You were saying that you shouldn't be focusing on perhaps taking a farinograph and making, turning it into a mixograph and refining current technology, like what you've done with the RVA and maybe that's not a direction that we want to go as a possible idea.

++ If you can ?? points came up this morning about handling. How will the handling system evolve if there is more segregation or IP. Because if critical ??? going to become more of an impact and there will have to be changes in handling system because tests will have to be done from the farm gate and every point the load is transferred there will have to be samples. Now whether that testing can perhaps be done on site or be taken to a centralized location, depends on *?tactics?* to some degree. If it takes 4 days to move from a producers bin to an end user, you've got 4 days in which to do the testing. On the other hand, if it takes 2 days to get to the test lab, there are all sorts of factors in this, so I don't know if there's a single answer, that's why I think there needs to be multiple technologies for any single test and maybe one that gives you a reasonably good vantage to identify the grain and then there may have to be one at the point of delivery that pinpoints the number. And they may have to be related.

That's what I was suggesting earlier; initial testing might be used at the elevators to do something different but definitely related to testing later on in the handling.

++ That way too you tend to minimize your expensive testing you only need one of them. Because at the elevator, no matter how good the tests are, I'm not going to invest a hell of a lot in it.
== That's right. You're probably not going to find many $500K machines at the elevators…
++ I was thinking about $40K
== If you can get in the $10K to $15K you're credible

Ron: we need to elect a spokes person, any volunteers?
I nominate George.
George, you're it
Ron: anything else we need to put with this first technology list

Yes. I'd like to ? insoluble polymers. ..thinking about an inexpensive centrifuge, spin something down…

One of the thing we've been saying for the 3 to 5 years is the molecular distribution of these proteins is very important to the kind of product you want. I'm sure there's a given set, a given distribution set of very good ?? end products ?? how these interact; but I don't know the technique right now that gives all this. We're looking at multi angle laser light scattering - lots of different techniques but nobody really has something that fits the theory.

What about insoluble polymer thing? That's not something you could use

Well it sets up pretty quickly, the first one takes a while but after that you're taking them out every 3 minutes.
Why wouldn't you put that down there then? If you're saying that it relates to…
But you're saying you can predict that with NIR
But if we don't put it down there; I think it needs to go down there
That might go under existing or something like that though.
I don't think it's something we're using in the…
Not for breeding

Ron: but if we use the criteria that for the test and the wish list, we want it to be rapid…
Rapid was the last thing.
Ron: yes it was but you understand why he said it was the last thing, being in GIPSA, but when it gets down to it, the farmer that's hauling his grain from the field to the elevator, he doesn't want to wait in line any longer than he has to because he has to turn around and go back out there and empty combines. The elevator that's filling up the train may be loading the train because he's full. So rapid is really something that needs to be considered. It's not the top priority but it does need to be considered.

Of course that's why you've got the technologies. You've got the NIR predicting than you've got the method itself which could be done maybe at a further point.

Ron: so if you want to put that down as another possibility or existing—it's just not widely used?
I think put down all the possibilities you've got. You'll have some that fail and may have another one that works.

So let's interpret 'existing' as something in current use.
Right. So in this case it is not existing.
OK.

Ron: so we'll write down insoluble polymers. You can explain that one. Anything else? Do we want ELISA up there or do we want to remove it?

Let me ask a question. I understood the Australians did some work on using dough strength, indication stuff years ago. Are any of you familiar with that? Anybody try to look at markers for dough strength? Surely you folks would know about that?

Ron: ok the second group to talk about is variety and class. Variety and class existing technology?

Protein segregation; hardness;
I'm not sure about chromatography and electrophoresis, put that under existing or possible. I do it for FGIS, board of appeals and review.

Does the French system use electrophoresis to back up varietal declaration?
Anybody using DNA testing for this?
In *?France?* what they do to test is combine; they look at calibrations for Near infrared for protein content and
Can't understand conversation
Basically can combine chemical measurements with ??

Ron: so chemical and NIR?
Yes combinations of the two.
Is the DNA fingerprinting thing now ?? something different than electrophoresis?
Yes, you have protein based and DNA based.
Do we want protein?
He wants DNA based and he wants protein based. Depending on whatever technology.
Chromatography?
Chromatography and maybe CE (*column electrophoresis)* as well.
Put NIR as possible?
And I think chip technology—lab on a chip stuff--that's going to explode—it's going to give us more information than we want.

Actually I did some work 10 years ago, I think if you built a large enough database, at least of the thousand or so I have, you could actually identify 90% of …
But it would be pure varieties, you wouldn't be able to…
Right. You'd know if it was a mix.

The thing I haven't done is look at mid-IR, because it's an even more unique spectral fingerprint for the variety.

Well eventually I can see using something like CE to measure, where you'd have these fingerprints of proteins that are in there, but you could use that almost like an NIR spectrum and it would correlate with parameters like farinograph, so it would not just be a varietal identification but in fact it would be correlating back directly to ?? protein, gluten strength, things like that, but we're talking about

varieties…the thing I put up there about protein fingerprinting,?? varieties, that's maybe a little more than that

--Well you've got all that information, then your varieties…it doesn't matter.

I see that as down the road.

Ron: the next one on the list is ??? Existing?

The o'graphs? Yes.

Ron: Possibilities:

NIR
It's already done
With your current technology, ????????????

Breakout session with Roger Friedrich as moderator.

…sure let's talk about protein, but let's talk about a couple of the others first. Maybe 10 minutes each, then spend 40 minutes talking about protein.
At lunch time I was talking with Ken and I said, you know if I take these export data, everyone's using the farinograph for one reason or another. ?? numbers they get out of it. And then people are trying to match NIR's to farinograph, and ?? was there talking about how it goes up and down. I started looking at it and said, you know the farinograph measures something but do we really know what it measures? So if we're going to talk about it, let's be quite specific about what it is about protein , not protein characteristics or protein functionality. Not just some generic term of quality, because quality means a different thing to every one of us in the room. So I guess I'm suggesting, let's take one or two of the others of that top to and talk about those and get them out of the way and then come back and talk about protein because it might even give us a different view of looking at protein. We all tend to talk about the same things we go back to the same techniques…just a suggestion.

Are you considering variety is part of protein then? If you look at –
--I'd be happy to go with variety and defects or something, two things and then let's move to protein after that, but if you are going to separate variety based on that because if—
--well no, I think if you looked at the variance you'd see that it's a fair enough component with genetics, the variety's going to have a big impact and the environment's going to have a big impact on hard grain wheat all graded number one. You've still got a big environmental variance. So if you want to cut down the variance to deliver wheat of like quality, the first thing to do is identify the variety, then you've isolated the genetic component. And then you want to do tests to isolate the environmental component. Variety is overriding, and there are techniques to do that. It is the most clean chunk of variation is variety. And people are buying on it.—

?? but when the farmer brings grain (cant understand rest of question)
-- sure you declare it but if you declare it wrong you're in big trouble

can't make out follow up question
I agree. Variety is so important and I've been using the ?? mapping? system and I've been reading what you've been writing about total quality management, and total quality management starts ??? and that means that and I've been reading the Canadian documentation about ?? and that's going to mean in terms of documentation and people are going to have to move to a quality… Like, I worked for a large inbound inspection company and we were ?? everything. We had to report everything and ??

--I would suggest that the technology that needs to be addressed is how do we define the variety after the fact. Like the farmer makes his declaration, there's an archived sample pulled. It doesn't matter if

it's tomorrow, next week, or next month. At some point it goes to the lab and on a kernel by kernel basis it has to be verified by variety.

-are you taking notes? If we don't write this down we lose track completely.

-in a way my answer Steve is there's no reason not to do this on a kernel by kernel basis because the technology exists now and is in use now.

Is it quick enough though on a kernel by kernel basis?
// There's essentially very little time constraint. The actual technology can be done essentially in 24 hours, and the two situations I would suggest it has application, one is a sense policing, the ?? declaration system verifying affidavits—
-- with the declarations you don't need quick testing; you need confirmative ??
-- and the second place is in the case of malt barley for overseas while the ship is in transit.
** idea of a 3 minute rapid analysis because you don't need that for—
-- it's a different system.
++ and it's a problem at the elevator. You need something quick at the elevator because you are going to get the rogue farmer who dumps into the bin.
-- only once.
++ once is enough

// But you see you may be able to develop strip tests that may identify certain proteins that may be associated with most of the varieties that are declared. You want to do a test to rule out suspicion first and then you want to do.. so you might have 20 varieties that have a particular band ?? bad ones, either doesn't have it or does have it. You may want to type it just to see the chances are 80% this guy's right or this guy's wrong.
-- So are we talking about gel electrophoresis too?
// Well I think strip test is the way to go but it's not the only way though. I think strip test is simple; I can't see anything else working at the elevator. It's like measuring the pH in your ?? Put it in, pull it out and it changes color.

And on that strip you'll have the different varieties identified?

You may have about 10 or 15 proteins that are associated with groups of varieties and you may be able to eliminate certain varieties that are present, or say that these varieties are probably present. You don't know if it's this one in particular but it's within this group and ---
++ and if it ??? then I can't see it operating on a single kernel basis—
-- I really can't see the need of it. Obviously you implement a new system and there's going to be a steep learning curve, there going to be people that make malicious errors, there's going to be people who make inadvertent errors, but I think that if you go and ask some of the Australians who have had this system in place for quite a long time, and ask "what is the error rate or what is the rate of incorrect declarations, my sense from talking to Colin Wrigley and people, is that it is very, very low, less than 1%.

I think that what we're getting at here is that if you're going to do variety and sort of have affidavits as a system, what you need is an enforcement mechanism, not quality control. And an enforcement mechanism can take a little bit of time.

// The big problem we've had in Canada is the liability issue. Nobody wants to be a liability. If you want to set up something like that, you may want to use that system for delivering parcels of wheat that have higher value. So if somebody wants to go to cost ?? meaning that a parcel of DNS that's 10-$20 more a ton, that particular group that can afford to do 10 or $15 in testing is going to ?? and the person who doesn't want to do it, it doesn't cost them anything. So you almost need a parallel system.

-- clearly DNA based technology, once you have the lab, not considering the overhead, the cost of running a sample is a buck or two.

++ No, it's 100 bucks a plate for chemistry to run PCR in the real world. You might be working under government conditions but 100 bucks a plate. You get 8 samples on that plate. That's a full blown PCR with all the controls. And you're thinking about liabilities here? Buyer and seller risk? 100 bucks for the chemistry plus your overhead.

-- well the overhead certainly…
++ and then you've got to develop primers and everything else. You've got just as much time in a PCR analysis as you do in a protein analysis

Well assuming you can identify varieties in the US, where we have a gazillion varieties, are we still able to do anything other than to say "well, now we can IP wheat. Is there anything we can do to address establishing functional characteristics within a grading system so we can blend varieties or…

/* Well if you look at the spring wheat area of the Dakotas, and Minnesota they learned that …

// actually we are doing a program for a baking company where we sell about 200k tons per year. Part of it is a branding, they can advertise that they use special varieties. So what happens is that farmers buy into the program, and they get a 20 dollar premium and the bakery determines how they grow it and what they do with it, and you've got full traceability. If a farmer doesn't want to grow it, he goes under the current system. You have a trace of ?? and if it doesn't make the bakery's specification then it gets delivered into the normal system. So there's a protein range and a visual grade range. So what we're doing is isolating generic CWRS and out of that we select, and the only private cost is that selection process. People who go with the bakery have to use certified seed, they have to have paperwork for all chemicals they use, all written down. So we're running two parallel systems: our normal system and ???

/* Do you see that happening in the US at all?
// I think that's happening right now.
+-There's some going on in the US and there are some grain companies willing to talk about some of the factors we mentioned today. They're not willing to give any guarantees but ??? it's better than it used to be.

// All these companies say it's variety ?? First you've got to identify the variety and then we worry about other things like the falling number and protein. Without the variety, it's futile.

Can't understand question

// They write up a contract
++ but you've already got the legal position in place
== has nothing to do with it. It's strictly a private agreement between a farmer and a company, we're not even involved.
Well you do their testing for them.
++ our experience in the US is that there are rogue farmers, and that's not a nice ??, it screws up the system when you accept affidavits.
// it's happened and what happens is, do it once, don't come back. I won't buy your grain anymore.
++ well unfortunately, the whole elevator is contaminated and 12 months later we're still trying to clean it out of the system.
-- well there's differences, I mean obviously, for example the GMO issue then the contamination is serious, but if it's a poor quality hard red in a 1 or 2 or 10% blend,

Roger: Let me interrupt, you have about 25 minutes left.

Just a question. Thinking about a comment John Oades made just a few minutes ago that the country elevator may the last part of the chain that sees testing that we are looking for, how can we apply this to inspecting vessels that are loading 25000 metric tons. Is there something we can do on

varieties, thinking about how many varieties could wind up on a vessel? I think variety ID is a good avenue to explore but relative to inspecting grain.

// I did want to interject that I was reading a paper from the Australians, and cost obviously is something that I need to look into, but they basically are using about 3 different, and I'm not big on PCR necessarily, but these guys are using about 3 PCR reactions each of which has 5 different primers to identify 5 different genes or anonymous traits and they basically developed what essentially is a barcode and identified every single one of the 36 major varieties individually uniquely in the Australian production. So it seems like the technology is there, it can be done, and maybe it's still expensive

++ NO, what's there is the R&D is there. The technology is not there. ?? company has gone out of business trying to make chips for this sort of thing. This is not a trivial thing. It's one thing to have the R&D and to have a research paper, it's another thing to turn that into a practical application to use in the field or even in an export lab. The money is there in the medical business, but it's not there in agriculture.

// these would be PCR things , maybe making the technology, maybe one of the researchable things for the group to consider is how do we get this from a cost prohibitive to a cost effective place. We've got 5 years, 1 year, 100 years, or it's never going to happen. I don't know.

++ actually along that line, if we can break it down like that, then there's probably only 4 or 5 proteins that are identifying those traits. That's what you are doing, you are coding for protein so maybe one strip with 5 different proteins will separate all the varieties.

// Could be I mean in the case of say partial waxy and non waxy, maybe one protein is enough. If it's a matter of sprout or no sprout, probably one is enough.
%%Maybe half a dozen is enough. There are other techniques instead of antibodies for tests. There are a group of technologies called "?? technologies" ..something is binding whatever you've got of interest. Couldn't get next sentence But if all you're measuring is 4 or 5 things, saying "OK variety 1's got some 1's or 3's or 5's, then we've got a real chance of relatively inexpensive test kit available.

But aren't you ruling out the possibility of admixtures?

Turned tape and didn't get question

…the cost must not be too prohibitive
no, but depends on what the premium ??

-- We know there are potentially technologies that we could use to identify varieties. And there are certain technical constraints involved in developing them. Is variety something in a pull market that is of real value to our customers? and I guess it depends on the test you are looking at. If we're talking about sourcing, I guess I'm ?? by it because I've had some ??? market?; at the moment then I don't know.

-- I think the answer to your question is, somebody is going to have to do a heck of a lot of education of our customers in order to be able to use that information effectively. That somebody may be US Wheat but it may be the Canadian Wheat Board. It's so scary to me because I spent 15 years deliberately avoiding the issue of variety because I knew it was something I couldn't really talk about in the export markets anyway. So I'm thinking I better get a real serious take on variety.

-- ?? talking about identifying all varieties. Could we not identify premium varieties?

-- ?? ones that could attract a premium price, or ones that the market's driving ??
-- Value added.
-- Or value removed ones which destroy the market; they reduce the value so much that the cost of ??? ;

-- Or varieties like ???? which have absolutely wrecked our spring wheat market in South America.

-- I wanted to stay back but I've enjoyed hearing this conversation. On the variety issue, don't be stuck on the fact that just because we can do variety or you can do it rapidly, at the point of sale or at export, that it's going to be used on a certificate. I mean, we'd have to change our law to do that. ?? those liability issue up there. We can't even do that, put that on a federal certificate. However, ?? a lot of information we could put on a certificate that would help the buyer, and I think that needs to be encouraged.

-- You couldn't even design a separate certificate that says, "this is predominantly variety such and such"?

-- There's a docket out right now on removing the prohibition against reporting variety.

-- Well OK. That's so being addressed. But still; it doesn't necessarily, because of the education part we talked about. So when we tell them, "you've got varieties x,y,z" what does that mean? And you've got a big education challenge.

-- ??? telling them what kinds of characteristics they're getting.

-- I mean in US Wheat Associates, collectively, we have a tremendous self education process. Our folks overseas aren't anywhere near as technically capable, as the people in this group, who understand why there is a difference in varieties.

-- see that's where I disagree. I think you'd find that many of your customers are more sophisticated than…

-- no I'm talking about our staff.

Many talkers

That's true, you can have all kinds of mixtures but you may??? have a predominant mixture and even that will give you some information.

-- and it will change from hold to hold. Because they change silos that they are loading out of.

-- I think you've got to cut back varieties from 200 ???? specific IP thing, where excellent varieties. The other one is to look at identifying varieties with particular quality characteristics, and you made the point about variety xxxx in the South American shipment. And what you may want to do is identify varieties where you have farinograph stability of 10-20 minutes not the 2 minute test.

-- Every situation is going to be different. There may be customers where the most important thing is it's partial waxy. There may be customers who say "I can't have any high PPO" or sprout. You can't make any blanket statements. I think that if one is able to identify varieties, you've gotten the first big chunk of variation to deal with. There's still going to be the environmentally induced variation to deal with.

-- before we quit can I just ask that we say something about gluten quality or something. I think we need to report that you can test it. I will stand up and say "by golly we need something with which we can get some kind of an indicator that the customer will believe in that indicates gluten functionality."

Something about using FT-NIR, and largely because if you look at the NIR we're using now, we're looking at broad bands and with the FT you're looking at very narrow bands, and you get a lot more information, and I think you have a greater probability than ????? protein detection.

I can explain for my colleague in western Australia his hesitancy to adaptability to using NIR..

-- you can put it down.. ?? has done some work on this and we've always been slowed by the good chemical data that can come from a lab where you use SDS-PAGE...
-- the problem is the primary method for the farinograph doesn't exist. What does the farinograph measure that you can refer back to a reference or primary method, so what you're dealing with is a secondary method and a secondary method.

-- can we move to the next method (*can't make rest of question*)

Well I guess all that that's portraying is that different have inherently different gluten strength properties and that correlated quite well with sedimentation volume across proteins. And we've seen similar things in the hard red and the hard white but this was a more acceptable data set. These samples were drawn from multiple crop years; we just went into our quality testing data base and just started pulling out …yeah clearly we had samples that went from 8-17% protein, but the salient feature is that we recognize that there are stronger and weaker gluten wheats out there; we see it in the PNW and in all of our classes, and I'm sure you do too and one challenge we've given ourselves is how can we get at a bunch of that variation and it seems to us that the sds sed test with all of its limitations does a pretty darn good job of making some significant inroads on that variations.

-- just out of curiosity, would you see these kinds of relationships in Zeleny sedimentation?

-- I'm not sure but that we've run very few if any Zeleny's and I can't really back this up with data, but my sense is that the detergent helps differentiate the gluten properties.

-- I would have expected to see it in the export cargo data and I haven't but that is based on Zeleny.

-- SDS is a routine screening method. We use it the lab every day to screen hundreds, almost thousands of breeding samples.

-- in terms of technology that could be either modified or automated, to me this has the best chance for any existing wet chemistry technology of being adaptable to the sort of constraints we are talking about. Now you can our small mixograph (can't make out the rest)

-- One of the problems I've run into is that there's a great amount of variation, well maybe that's too big a word, but there is quite a lot of variability in terms of repeatability of this test; and so when I try to develop NIR secondary techniques for sedimentation volume, I am fighting the repeatability of the test. So my thinking is that if you could somehow tighten up the controls on this to somehow make it more repeatable, it might be that the NIR would be even better at predicting it than it has been, so far I have shown OK results, I wouldn't say great results but OK that maybe a breeder could use, but no one else. R squared of point 8 or point 9.

-- maybe we ought to exchange a weak medium and strong wheat sample and grind it and run 10 or 20 reps and our own in-lab; maybe there's something that each of us can do to tighten it up. I feel ours is pretty reproducible.

-- and does this lend itself to something very rapid?

-- what I have envisioned is that to have something that looks very similar to a falling number; as quick; as simple; obviously won't use exactly the same equipment.

-- what's nice about this is this crosses the boundary; this isn't just a hard wheat test; for the official system that's what we need-something that does soft wheat for soft wheat customers, hard wheat for hard wheat customers; that pays big dividends for working in the official system.

-- as we've said, we measure protein content very well; the next chunk we don't have is that whole thing called gluten quality or gluten strength. Whether we assess that with a farinograph or a mixograph or an alveograph or what have you; and clearly low protein samples don't differ much—

there's not enough protein to give much differential expression; but when you get high protein then you really see the effect of that gluten.

--I 'm going to play Bert D'Appolonia for a minute. We think a lot alike sometimes although he says it better. What happens when I put a hard red spring wheat in something like this? Do you get any sediment at all?

-- well yes, the numbers that we have; we have some really outstanding high quality hard red springs in Washington state now; and some of those at an equivalent of 16 to 17 protein, were going well over 300 almost 400 sedimentation volume.

-- Do you find it correlates with anything besides protein content?

-- That's probably where we need to do a lot more research. Clearly there's a good correlation with things like loaf volume. I think we need to do a lot more work with things like farinograph stability. It's tough to build up a big database on farinograph stability because it's so hard to run that many farinographs. But that's something we need to do to underpin the whole concept.

-- the correlation to loaf volume..??? less than point 5

The groups returned to the plenary session and reported out the ideas generated. Based on those ideas and the ensuing discussion, participants were assigned to new groups based on the expertise of the participant: protein expertise; instrumentation expertise, etc. All three groups were given the same general question: ***How do we develop the promising technologies needed to measure the ranked quality factors" from the Matrix?*** but each group was given a specific areas to focus on such as protein, starch, etc. The original plan called for the discussion would go for about 1 hour that day, adjourn, then continue for another hour the next morning.

Because one group was assigned selected areas which were not in the top ten list, there was a bit of struggling at the outset, however all groups got their discussions on track and they were productive. As the groups had already worked very hard, a decision was made to end the session slightly earlier than planned and restructure the breakout groups and their topic assignments for the following day. Again every attempt was made to transcribe faithfully, within the quality of the audio and the speakers aural considerations. The transcripts of the truncated discussion of two of the groups follows:

Breakout group with primarily imaging/engineering backgrounds, moderated by Roger:

Roger: Technologies that are out there for imaging. Are there any that are developed or being developed that you want to talk about? That could be all three of these or any one of them in particular?

What kind of resolution is available in digital imaging systems nowadays. What I found on those slides didn't look like it was all that whippy.

What category, or what do you want me to call it? Digital imaging or do you have some specific

…??? singulate it somehow and image them one by one. OK?

Well and then there are also results where they look at the whole, a sample in bulk, you follow me?

Yes.

Holy cow. There are some mathematics going there!

Well in that case you are not trying to segregate the kernels.

Cannot make out discussion

I think the answer to the question about what resolution is that, and depending on what you are looking at, is why get more information than you need. Depending on what you are looking for, figure out the minimum amount of information you need to make a decision.

--If I understand correctly, you are saying that you can hurt yourself with better resolution and you want to use the minimum resolution you can get away with. Is that right?
Yep

Roger: Any other discussions or questions?

Something about spectral imaging

Our philosophy on spectral imaging is that we might……. before anything else we can identify those 2 or 3 wavelength that can be used in a real time system specs.

Cannot make out most of discussion. It is about insects and finding infestation damage by imaging

Is there anything anywhere else in the imaging area we can put down?
…in cereals we are working on light systems for vitreous kernels and working on modeling ???

Are you doing anything like fusarium?
We were. Well. We were going to, but it's a low priority.

Question about vitreousness

Vitreousness, again, to some degree. From a research point of view, we're not ??? quite there ??? the necessary ??? We want to… We've identified the high. We're prioritizing sort of the ones that we can see that bring a result in the. You know, taking on something that good ??? that you can do something else with, that's deliverable in a reasonable time frame. So. Research priorities and commercial priorities aren't necessarily directly aligned, and I think there's got to be a bit of reality between the two.

Are you using a gray scale or color?
Our original system for durum used a gray scale but ?????????? The reality is we probably aren't going back to gray scale because they're not getting bad results using color image. Again it just has processing.

Virteousness doesn't enter the picture for CWRS then?
It does but it's not such a priority

Question—can't understand

The grain handlers ????? finding profiles on things like mixing and ????
And what was your time on that?
15-20 seconds
In bulk? How do you present the sample?
????
--Basically what we identified is things like ???? In fact we know there's a direct correlation between kernel shape and size, and we know that certain physical defects, black point, smudge, some of the

molds, insect damage ??? One of the questions I think we need to be challenging our inspection stations with is what are the different sorts of damage ????? again by ?????? and again some degree of ???? The critical question is how important is it to know why a thing is damaged. And then, we haven't got a clear answer on that. If we don't need to identify everything down to ???? we haven't ?? inspected it because they do that now, you know whether its midge or ?? insects or whatever. Is that really important?

--I would argue on our (US) grading system that some of those damaged kernels that are graded as damaged in our system, oh they might worth a penny discount and some of those damaged kernels might be worth 10 cents of a discount. And some of them are anti??? maybe but none of us But there are some that are much more devastating than others as far as end use quality.

Discussion halted by McCluskey

Breakout group composed primarily of protein experts, moderated by Tim Blackburn :

Tim opens: Let's pick out a particular quality factor/ a particular technology and decide how we are going to recommend how it becomes developed, or promoted or perfected or created or whatever.

Make a comment that someone at USDA has tried to come up with these tests for close to 20 years with mixed success. One of the difficulties I have found is we'll come up with an idea for a test that's needed and at a research level, we'll go into a number of years developing that test, I'll use KB for e.g. What we need to be very careful of is what do we really need? I think that's the hardest part is picking out what's going to be important.

Is there way to pick out in advance what test will be used or is it something that will be obvious after we have played with it for a year or 2 or 3?

I think we can have some idea of what is the need now, lets say we talk about variety, what do we need in terms of classify things. That drives how the test is perceived etc. But then what is the purpose of the test, what's driving it, then we are more likely to come up with one that's useful in the long run. There are many more useless tests than useful ones

Tim: Other thoughts?

6 or 7 yrs later, protein quality is still our #1 need now in our wheat market. Nothing changes my opinion of that.

Tim: Ok is that where we want to focus then, right now?

Protein performance?

I think it is those characteristics/traits we think of in terms of dough mixing/rheology, batter viscosity, end product texture.

Do we want to put some of those down just to give some reference point? I think you're absolutely right, what you're trying to measure, It's difficult to say well go fix it.

There's no one perfect way to do it, to get at it, even though we recognize how important it is, because we have all these different o'graphs. If there was one easy perfect way to do it we wouldn't have 5 different dough mixers.

The 2 primary o'graphs that are used marketwise are farino and alveograph. 3 basic tests from each

P,l,w from alveograph. P l ratio I've always heard is a 2nd derivative of other 3. For farino it is mix time, absorption and stab.

Wet gluten active in market.

They're reflections of what people see as characters of protein performance. Ways to try to quantify protein performance.
Has anyone been able to figure out molecularly why one protein will give you different performance than another, i.e. different shape, form amino acid comp…anyone have an idea about that?

Lots ideas but too complex to discuss here

People talk about numbers of disulfide bonds, HMW glutenins, length of repeat sections, lengths of individual monomers, N terminus, C terminus all kinds of theories

I mean if you did or didn't have an enzyme that would be one thing but you have this multitude of storage protein interactions and to get these kinds of characteristics they become large multimeric structures and they are cross-linked and things like that

Yes and you have got about 100 or 150 of them

Because it would be all relatively straightforward if they knew the molecular detail and you could look for that aspect of it. Other than that you are stuck with functional things of it and you have to do the functional test that says it has this characteristic. Unless you could relate that functional characteristic to a molecular detail, that doesn't sound like they are there yet

7-8 yrs ago someone came to us and said they had molecular modeling of gluten and that when it was in this particular molecular configuration then it gave the kinds of characteristics that were useful functionally and they wanted us to make an antibody that would recognize that particular conformation and that is something that I would say would be feasible then if you had an antibody like that you could put it at a silo or grain handling facility because you could have a very simple quick assay like the GMO kind of test. But if we don't really, and this was some number of yrs ago, and I don't remember if this individual, but I thought this was a USDA person, had come to us and said they had this idea that a particular structure related to or correlated to functionality, if it really isn't that far along yet, you couldn't do that yet. If you could correlate structure with function, then something simple could be done.

There certainly are certain HMW glutenins in a fairly small number that are generally perceived as being better for certain performance and others that are poorer for certain performance. I know the Australians have tried to make antibodies against say 5-10 vs. 2-12, been moderately successful at it. And that would give you the first bit of quite a bit of variation, I don't know if it would pay for itself.

This gets to the point I was trying to make earlier, that it's a difficult one for someone at the biochemical level to say OK how can you develop a test when you don't know what your analyzing for. And here what we've done again…performance characteristics, but let's say that we've got a few glutenins that we can make antibodies to, how far would they go for predicting the quality you want, and how well would they be accepted against the traditional performance tests. All of those come together but as a test maker it is very difficult to think of getting into one unless you know…

A problem is you have the environmental effect, so you can have all those proteins and the environmental effect is almost 50%.

Do those environmental effects also affect protein structure? I don't think so, there are some differences of opinion there, some people think there may be some slow translational modification there, that add to the environmental effect on protein, which makes some sense, but there's very few people who have found them. I had a grad student who found some on 2 different wheats but there's nobody else who's been able to find them. And they were very low levels.

I think the reason these are prevalent and have persisted in the market place is in sense they summarize a very high number of interacting bio-molecules and what I've been thinking thru with the SDS sedimentation test is it similarly summarizes the whole bucket load of things we that can't always put our fingers on all them. But it is a heap simpler than these dough rheological methods, so in a sense it is kind of in between the two worlds, trying to gain practicality while still holding onto as much information as possible.
Another topic that came up at our other session, is that whatever we come up with as a shortcut or a simpler way of doing things, it has to be expressed as the equivalent of such measurement as farinograph for example. Or if someone wanted to do a farinograph, it would confirm that it was either A,B or C for example, if you were talking about classification structure.

One area I don't really know about. Say farinograph test for environmental factors, are you seeing environment caused differences in farino results will also show up on .. factors…. If you grow a variety in different areas, it seems like there's got to be something besides the dough characteristics that are affected by the environmental.

Yeah I've got some data on that- I didn't do it, Bob Bequette did—he compared 5 cultivars dry land vs. irrigated different protein content different water absorptions, and he had mixo and farino data and the interesting thing is that with the farino he saw very little difference between the environmental effect and also has loaf volume and crumb grain characterization, and pictures of the loaves, and did see it with the mixo; definitely the mixing time changed and you could see the decay in terms of the stability. And with the farino you didn't see it until you got into the higher protein levels where you had better quality lines.

Are you saying that farino did not correlate all that well with environmental conditions?
It didn't correlate that well with environmental conditions or with the quality that was being determined in the baked product

But the mixo was better?
Yes the mixo was better.

But it's difficult to get water abs with the mixo. It takes more expertise whereas with farino you come to the 500 BU line. It would be nice it they'd do that-it would be easier.

The other interesting thing is that there are 2 different bowl sizes with farino and you get diff abs depending on which size bowl you use; they all have the hocus pocus factor but they are also empirical, valuable tools and that's why people use them.

If a functional method is what we're left with, then how do you bring a functional method to the grain elevator? That s what it boils down to, if we don't have anything molecular we can point at, so we are left with doing it empirically,

Or finding some other method that gives us predictability, right?

It has to go back to whatever we measure.

…then you've got a marketing problem. Bingo! Very often we look right past that, as first we did with SKCS, we had technology and lots of promise and a damnable time trying to get the market to embrace it.

That's a good example of why we should not get hung up on one technology. It may take 2 or 3 methodologies, …. to give us the information that we have to do to give us that thing that people want to relate to. I hate to use the same example but the farinograph characteristics. It may take 2 or 3 other tests to , OK, this same information is giving you the same information that you get from the single farino test.

To tell you the truth, we have the first 2 and biggest chunks of this, the first is class and the second is protein content and obviously there's still an unacceptable amount of variation left. Actually …has the first two biggest chunks of it….hardness? Absolutely and absorption. I mean if we were comparing equal protein HRW and French wheat, that hardness is a big factor.

… so they do the protein, the moisture, W, the ID and the wet gluten, zeleny---(talking about a euro system here)

Is that used for segregation? Or for price and contracts?

And the combination of protein and W value indicates variety. But of course they have contract growing.

What kinds of correlations do you have? I'd have to find out the exact number. I mean for the variety vs. those two components?

I mean it certainly strikes me as something that GIPSA/ARS should be evaluating.

Some places class by protein so it's not the same situation as here.

They're using varietal declaration too which speeds up the process a little bit about what you can expect.

Must be sure that we keep in mind that at receival point there will be mixing and at the next point in the chain there will be more mixing of varieties.

Is Foss prepared to sponsor a validation research project in the US? I'm very interested but guardedly skeptical but I think it's worth looking at to see if it was true.

Maybe more basic and I've asked and we pushed real hard for an alveograph calibration.. is that something that's generally available if someone would like to add it ?

Wet gluten remains focus of calibrations.

Factors France is capturing: protein & moisture via nir

If the goal of the session is how to go about doing that, wouldn't you have to develop the calibration for our kind of wheat? So one could envision a collaborative effort between, I don't know, someone has to provide samples to generate data,--FGIS…

One has to select which samples to include, and try to minimize… we cannot collect samples randomly. ***Discussion halted by McCluskey***

> It is clear from all the transcripts to this point, that the discussions were very engaged, lively and very technically oriented. The many perspectives of the participants are apparent. It is clear that there are technologies which can be explored/exploited. These include NIR, ELISA and chip technology, sedimentation volume.
>
> *The meeting planners restructured the activity for Day 2*

DAY 2

The workday commenced with a presentation by Rich Pierce on GIPSA program for collecting samples for characterization by NIR and numerous cereal chemistry methodologies. Following this, we presented the revamped format for the days work. We divided the main group into two groups rather than 3. Each group was given a specific set of questions. The question for and discussion transcript by each group is presented below.

From the list of the technologies, how do we develop the most promising approaches to characterize gluten?

- NIR
- Ultrasound
- SDS
- Mid-IR
- Raman
- ELISA
- The O'Graphs

The discussion transcript, moderated by Tim Blackburn, is presented here:

Tim:…energized around what we talked about, protein characteristics protein performance; we discussed several different kinds of technologies that will help us define protein characteristics then got into a good discussion about things that are happening in France. ?? talked about some kinds of interactions and collaborations to learn about what's happening in France, things that might be useful for us. And that's where we left off last night. Since that group is largely expert in gluten, protein, we thought we'd ask you to look primarily at the characteristics of gluten, and look at these technologies that we identified yesterday as a group, that can be modified or adapted used in new ways, to help us develop the most promising sources to characterize gluten. We thought we would focus on that as a group for the next 2 hours. That is looking at these and perhaps other technologies, to see if we can come up with approaches to use them how we can better, use them differently to identify the characteristics of gluten. Does that make sense?

The most promising in my mind is rapid. The focus should be on the most promising that is accurate, rapid;
That also we can accomplish this in 3 yrs. Is there a way that we can look at this from two perspectives; 1) something in the interim that we could incorporate in the next 6 months not the next 3 yrs and then look at something that's long range. As far as the interim, taking an existing technology and trying to modify or change it, that might be applicable in the system.

Dave Shipman hopes we will talk about interim technology—something we can get out there soon.

I think another reason we haven't addressed?? And we're looking at dough strength, and identified NIR, and Brad is working with NIR on hrw, I think we're missing the boat because HRS really is what we should be focusing on. I guess one thing I'm kind of wondering, is whether, before we get too compartmentalized about what people are working on??…because again the opportunity to realize, to look at …for instance, and see what's happening, I think HRS is a much more logical place to…

…regional biases of the united states, whatever the solutions are, that come out of this, have to be approached from the standpoint that we have to address the entire wheat industry and not let

regional influences, of crop improvement programs, etc, etc, skew the effort toward one type or class of wheat at the exclusion or diminution of the effort or attention of the other classes. We've seen this before and I'm a victim--we know that was one of the problems with the SKCS. There was too much emphasis on the hard wheat and the ?? soft was turned around…
Oades comment—can't make out

And I do recognize the fact that we do have regional concentrations of expertise in wheat types—Fargo and Manhattan are a good example, but that doesn't preclude us from approaching that entire problem. And one other comment-I have to support Bert's remark about interim procedures, because-Rich help me out—doesn't by design the calibrations that are done on a chemometric system require the inclusion of at least 3 crop years?
** In looking at a robust calibration, I'm not sure there's a hard fast requirement for …

My caveat is, in the current effort we are talking about, we have to go back to the drawing board to start looking at the inclusion of other chemometric models. I'm sure the spectra is ?? but I'm not sure that the corresponding physical data, regional farinograph or alveograph …?? So the caveat being, OK three years from now, when they want to release this, we are gonna have optimistically 2 crop years of data in it. Don't put ourselves in the position of having to catch up, with an early release of algorithms that sort of work.

Is this where funding can be used such that you can fund someone from the HRS lab, a technician come and say come and work n Brad's lab to do the Mid-IR material, so that the funding is facilitating the spread of that technology to a wider class of cereals?

In a ?? regional wheat quality lab we are dealing with thousand samples but those are all ???

What I'm getting at is that we have regional expertise. We also have regional hardware. Not all labs have, I don't think, have Raman spectrometers. So I think again, this falls, I'm not sure who's executing or doing the planning on this factor data set, but for lack of having a name to apply to it, I'll say since Rich is the one who presented the information, I think he's the one who should get it (or see it or carry it) through, are the ones who are going to carefully select the samples.

And I think that what we've taken is kind of a preliminary look. The easy thing to do was to say "well lets look first at the instruments we're already using out there, and whether or not that was good. And I don't know
if going into this project we thought , yeah that's the instrument we want to use long term. …and we now ..NIR's .. which may not be the instrument either. so I guess that partly the response is we're still learning—we're still relatively new to this process, compared to some of you who have been working all your life, and so I don't know if we've said this is exactly where we want to go??? And we are considering the protein? work ...throw all the wheat classes together with the models …I'm not sure whether that gives us good enough prediction?? let's say HRS prediction.

the only concern I've got is, on one hand we've got a fairly conservative approach-we take the first crop year and take a look at it and see what-whether it works. Then that leaves you only 2 years which means that for the 2nd year, you may get retains from this crop year, maybe, for the other classes, or you are going to be putting all your calibration eggs in one years basket. So that's why I think it supports Bert's comment that we need to prepare for or at least come up with interim solutions if we can't come up with an electronic solution.

Yes I think it's true definitely that what we are doing now is actually convening a study of so called modern technology, using NIR …

Yea but with all due respect to the FOSS people, the NIR is not going to be the total solution. Everybody can accept that. And I see Jan-Ake over there nodding his head.
Yes, it cannot do everything.
OK. So that's just a caution. Dave Shipman's request to have this ready May, 2006 may be a little bit optimistic.

Well, there's other things on the list that we'll certainly have to have well positioned by then.

Tim: OK, so I want to make sure that we hear from everybody as far as what's on your mind right now as we begin this. I've heard that we need to look at this as an interim, and also the longer term; the same technologies that can be used now or modified and adapted to be used as an interim; we can produce some very quick results; and then efforts that will be set on longer terms, maybe 2-3 years, may even a little bit further out. Is that what I'm hearing so far? Obviously we'll need 3 crop years or whatever those types of things could be worked on. Other thoughts as we begin this process that you want to get out on the table, the kind of beginning point.

Well the SDS sedimentation test, one of the main reasons I guess I'm a proponent of that is that, one thing is it doesn't need any development. It was developed whatever 30 or 40 years ago. So we can hit the ground running. It just needed some automation/gadgetry? so I will be working on that straightaway-have been working on that. That's clearly within the timeframe???

Tim: We'll come back to that and make sure that's up there- something you can use immediately and in the near future.

I think whatever we look at …there has to be something related to farinograph absorption. Cause that's what you're going to see.
That particular technology is used internationally, understood by everybody?
There's others as well.
Other thoughts, points you want to get out, because I know we haven't heard from everybody yet?

Does anybody expect that there will be one technology that will be applied or whatever…algorithm…

I just want to follow up on what Bert was saying. There is a difficulty in trying to relate farinograph tests to anything we can come up with, simply because again going back to what we were talking about yesterday, we don't know exactly what on a biochemical level what a farinograph is measuring, so it's going to be a tough part if you do have to have that standard, I agree with … I just want to point out that it's going to be a difficult one to make those sorts of correlations.

I appreciate your comment. The point I was trying to make is that the export market realizes that … 15…you have to someway have some relationship to that…
I agree with you…
I guess I would add to that, that there's nothing wrong with trying to relate that back to farinograph stability, or mixograph or whatever, but each of those instruments, the units of measurement are unique to that instrument, they are not transferable in terms of rheology, to any other thing, so I guess I would add, in terms of say gluten strength, we should be looking at recognized fundamental measurements of rheology like a rheometer, that would have some benefit to science, not just cereal chemistry alone.

Yea that comes back to a comment I made yesterday, that quite frankly we need to look more at that relationship between chemistry and functionality, and I think we really need in agriculture to be more aggressive in competitive grants program… that's why the petroleum and chemical industry have made such strides in advances over the decades, because they understand that functionality…chemistry and there's a relationship between chemistry and functionality…and we really don' know what makes…and what makes some of these technologies work. I think that's part of it, of course we're looking at shorter term here, so whatever we decide might be applicable within 3 years, we may not know that by then, but I think these have to go hand in hand.

Under short term interim, ongoing is the SDS, and we're adding more gadgetry, bells and whistles.
Now what are the characteristics of the gluten that this focuses on?
I guess it' sort of a summation of the swelling properties.
Some indication of the amount of gluten involved?

It's highly related to protein content but also protein quality. It's highly dependent on the genetic quality, for example, with our club wheats, we designed them purposely to have essentially no gluten strength at all. So even if you go up to 14, 15 or more % or more protein club, it still doesn't develop hardly any gluten in it-it has no strength to it. Whereas on the extreme other end, would be like the Canadian extra strong.—too strong to go alone but great blending wheat.

One other thing on SDS, …customers talk about Zeleny, we talked very briefly about what the difference was You may want to rationalize that somewhere so if Zeleny isn't typically different but is adaptable, we may want to go that way rather than SDS unless there's a clear reason not to.

Well there's a wealth of information on the equalization…
I know the difference…
of the Zeleny specification in the early 1960's if one wants to review that paper, and in the case of HRS in a nutshell, it's not applicable to HRS.

Actually, our greater theme song is that once we have the hardware, a person could run SDS, or Zeleny or we would hope even SRT's, so it should have, we're really gunning for the broadest applicability, the widest range of test parameters.

Tim: There was a characteristic you mentioned I didn't capture, something about protein or gluten.

You know I mean it's really a predictor of protein performance, gluten performance…

Tim: So you recommend it for soft wheat?

Oh absolutely, because really I think what we're witnessing is a delineation certainly in the SRW market, with very weak traditional cookie types and then the stronger, what I call baguette types; but we need something where we can differentiate those because they are all the same class. And similarly in soft white, we have very weak types and we also have ??? soft whites.

Basically when you don't have enough protein, at very low protein it doesn't matter what you have, it's mostly starch, you don't get much functionality, you just don't have much gluten functionality.

Tim: Do we need any more discussion about SDS and what it will be testing for, is it something that is going on and will be focused on more in the immediate future. If not we can go on to another technology or..

I have a quick question: in terms of sample size, how much sample do we need?

It's quite small, there is a standard SDS test that uses a couple of grams and a 100 ml. cylinder, then there's a micro test sed test that we use in my lab and I think it's a half a gram.

So this is the ground up sample or flour sample?

Right, what I'm planning to do is try to adapt so that I will use just another aliquot of the meal ground for the falling number test, so it would be the same sample ground for the falling number and take out a bit of that and run the sed test.

I guess why I asked is that any of these tests that require milled flour is going to be harder to adapt.

OK let's see if there's another technology from the list up here on the board or any others that can be used in an immediate way, as an interim measure,

Well I…mentioned yesterday that there's something like the mixograph where you can do it on the ground whole wheat and it's not going to be exact but at least for grouping whether it's mellow, strong or very strong, and I showed the curves yesterday, but it would take some work in terms of ..?

I'm not sure they need the entire mixograph. What we need is a mixer and a chart to get a curve…something basic. It would take some development work; I think if we get 250 inspections???

Discussion turns to money: I don't want to throw the money financial constraint in the works but if you did something ???

Well that kind of a number would be very attractive if you want to get broad use at the country level. I look at the 10, 12 15 thousand dollar figure if you are trying to get broad use at the country level. You get much above that then you get some rapid decline in their willingness to invest, for multiple locations at the country level.

Tim: what are the properties or characteristics of this ??

One of them the mix time and the mixing strength.

>>>still working on the alveograph?
I guess the reason I bring this up is ?????? to duplicate the farinograph?? In terms of mixing ???? similar types of ???
Well you know Australia's got that little V-arm?? mixer ???? that might actually relate to farinograph more closely..I don't know-I've never used it..
Yeah there are certainly modifications that…
Craig, do you know anybody looking at that technology?
I don't know anyone who has one..
It's called a V-arm?
Yeah it's got sort of a paddle shaped mixer as opposed to an orbital pin mixer

How long does it take to run…
I would assume on the order of minutes like all these things

Tim: Are there any other technologies we could use as an interim or short term test methodology?

Well we just started some very preliminary work with Perten, where we sent them a number of samples we evaluated with the farinograph, mellow, strong and extra strong, ?? look at them with some modification of the gluten index ??????????????????
One think with the gluten index, it does give you???? information relative to the characteristics of the gluten, whether it's extensible or whether it's soft?;

What's the range you are in?
Gluten index? Spring wheat you get something in the area of 100 or 95.
So what's the use? In domestic data from regional lab, they are all above 90-95

Tim: so are we saying farinograph is another technology for the short term or…

We need another technology.

Well I was talking the "Glutomatic"

Tim: Glutomatic? That's a technology?

Wet gluten is very important in the export market and you can tell it's got a good correlation to protein content

The other thing is that provides you with the wet gluten measurement.

I don't think we used it since that first year so that Wilda had to get them all ?? I don't even remember..

I'm thinking more in terms of, there might be a modification, something in the springs or whatever,

Tim: Anything else?

In defense of the soft wheat guys, we should put SRC up there. As another available technology.

What are your opinions relative to the longer term adaptation/adaptability of SRC to wheat hardness?

That's the question I raised yesterday at the session. Nobody's really tested it to any great extent.

From discussions with Charlie Gaines' group, it's really an indicator of how they can use the wheat as opposed to whether or not it's strong or weak or whatever; it's more of an indicator of the best usage for a given plot of soft wheat

As you well know, Nabisco's all over the world with that driving interest in the overseas market and technology, so if anything gets a little precious down there…(interruptions)
Well that's what I was saying, you just can't ignore that as a test …you standardize it, (then too many people talk at once.).
But I'm just wondering, should more work be done with a test like that on hard wheat…Craig are you working on…?

I don't use the full battery because I'm not that keen on the lactic acid one…but we run the … and we run the water … and the …

Tim: Anything else I need to capture up here?

I think I should mention that we are doing some work at the hard winter wheat quality lab (can't make this out)

I think one thing that's possible that might be derived from that is a screening for mixing time, whether they are short, medium or long mixers; the key there would be to try and find, if there are any, relevant overtones in the infrared, that could be used by the NIR rather than by a near infrared Spectrometer; the problem is they may be so weak in the infrared that…

Is that a short term implementation?

Well I think so. I think it's one of the next steps I want to take in the work that I've done too is to try and because I think it's applicable to breeders, even if we said you know lets get a cheap mid infrared detector and we work with our engineering unit, or work with, and I talked to John and Gavin last night …a company that we could construct something very simple that we could make use of bands that we have in NIR that would address that. This is following the secondary structure of the protein that develop as the dough is mixed over time, and of course the thing I was looking for was can you follow the rheology of the system and does it correlate with the actual mixing time of the dough as determined by the mixograph, and of course the curves had a reflection that corresponded to that, but you can also see that in, and this would be like at one minute, so at one minute or less, the flour hydrated, and you can already see as difference as to whether that flour was going to be a long mixer or medium mixer or short mixer. So in a minute or less you could have a test based on the mid infrared that will tell you whether that dough is going to mix long, medium or short. I think would be the utility for breeders but also for anybody else, and totally based n the secondary structure of the proteins as they hydrate.

And we have not done farinograph, just mixograph.
Brad would that relate to stability?

I think stability information is there, I just haven't pulled that information out, I was just basically looking for mixing time and that was it but I think it's there.

Tim: I've heard short term and interim technologies that are already being used and that are being adapted to get quick results. So far we have MidIR, SDS, Mixograph, Glutomatic and SRC.

MidIR, would you like to put it in short term?

Well I think the screening capability I mentioned could be short term, the other aspect …

Tim: So we could add that in as a longer term?

I think the screening aspect for mixing time would be a short term.

Jim reminded me that yesterday we were talking of the work being done in France with Foss and so on, NIR applications, that we don't want to lose focus of, so I will also put that under short term.

Oades: you really ought to check that out see what the adaptability is; probably the calibrations would have to be developed for US wheat; if they are able to get an alveograph W value, we really ought to look at that. Any market that's looking at French wheat or European wheat likes alveograph values. You saw that in the survey I did; that would be a critical measure that might happen fairly quick, although I have no idea how long it takes to build those calibrations.

Jan-Ake, do you know anything about the chemometrics used to build those models?
** Those are PLS.
-- All PLS?

(garbled)..there's a wet gluten calibration, that if you look at one?? you have a ???

One comment I'd like to make on this French group calibration is that they have an organization that the group they have doing the NIR calibration is part of a larger group that ultimately is responsible for the wheat quality survey in the country. So in terms of the statement that …"the samples that are coming in and using the calibration" they have a very tightly linked system, and I wouldn't want what we've got here, we've got one group over here …NIR, and I cant help but think we've got some real opportunities for getting more bang for our buck; particularly with these samples that we've already got the quality data on.

25 years worth of research and we're… Any other questions?

Assuming they are already small samples, we could probably arrange to get you a small fraction of each one of those samples that are gathered for crop quality.

Tim: all right we've been focusing on all kinds of short term and interim technology, should we focus now on something that could be developed in 3 or 4 years that could give us some payback- is that a logical place to go?

…in terms of NIR /Raman, this is in our CRSS research project, work that we plan to do regarding dough rheology, regarding getting a better understanding of gluten structure and how it changes in the mixing process; Raman in particular, I think I could count on one hand the number of papers by anybody who has even attempted to use Raman on flour, maybe one or two; it's a wide open area; we can say real interesting things about the macro-molecular structure

--you want to add one more hyphen and add mass spec up there as well; spectroscopic techniques is a better way to describe it--
Brad would these all fit in to the criteria we heard about yesterday? Is there going to be any difficulty…??
--You're talking about the rapid--

--Yeah..
--Mid-IR is probably the one that's most conducive to that line of thought, because that's been the instrumentation that's dropped significantly in price, that type of thing, it's always been fairly rapid. Raman that could be kind of tough…
--Both Raman and Mass Spec are being used in process now; they should be able to be made fairly idiot proof--
--I think that's just starting; there are some indications that these technologies are bringing the price and cost a little more affordable, and more rapid, but they are not quite to the point where???.

I wasn't sure we even had idiot proof NIR's.

What I'm getting at, OK, if you've got an in process application for this technology, the more typical parts you have to deal with in the delivery system for example, and that's not trivial but it's certainly a lot less than trying to rugged-ize the technology for industrial use.

Question to Craig about rheology…

I think these technologies or applications are the new phase of cereal chemistry, where we're really looking at the molecular level. We are no longer relying on tests for some typical response, we're now getting down to the molecular lever where we better understand what's happening. I think it's a very exciting area to be work in.

And this almost gets into that everybody was talking about--chemical composition. These three technologies in tandem probably comes close ?????
--Right. Very powerful matrix.
---something about mass spec.
However the advantage of Mass Spec is that it comes as close to a spectroscopic primary method as you can get.

If we're going to be linking mass spec, I think something that goes before that is the third one down, linking capillary electrophoresis, which is quick, to the mass spec. and that would fit in to that general spectroscopic analysis.
--this is not a $12K instrument.

Tim: other kinds of longer term technology?

I'd like to bring up something that George and I have been discussing as a subset of this and that's water absorption and using ELISA approaches for doing that. And we spent some time looking at a couple of things we might do for example, a multi analyte system that's very rapid; it's quantitative. The ones we've come up with looking at for example have been starch damage using alpha amylase, pentosans and protein content. I… think in the longer term within the 3 years of having something available as an ELISA methodology; and would certainly fit within the 5 minute range; and in fact, something… talk to Jim about, is the potential of commercialization, is having a sort of collaborative effort between for example USDA, university groups and key people in other countries like that, to make sure that (it's across the board?)

You mentioned water absorption, protein--what are the basis on which you'd measure water absorption?

Is that technology available to PPO methods?

I … no reason why you couldn't have monoclonal (prints??).

What is PPO? Polyphenol oxidase. An enzyme? Yeah.

And you just want to know what it's concentration is? Yeah.

That should be strait forward; so measuring the protein concentration by an immuno assay is what it does. So I think longer term there is a variety of things that could be done with antibodies. An antibody recognizes a 3-d structure--that's what it does. So if you could ultimately relate structure to function, and decide and understand that when the gluten has good structure, it correlates with good function in a farinograph or something like that. If we got to the point that we understood the structure-function relationship, you could make antibodies that recognize the structure and you could have your 3 minute task with no training right there at the elevator the way it is currently done, and you could say it either has that structure or it does not have that structure.
--The structure probably changes during mixing and that's where you're getting that optimum structure--
--yeah one of the most difficult things to say is …every flour has 5 chemical components that are common--the combination of components in the presence of water…--

I understand the whole thing is complex, there are all these variables that are associated with it, but, the fact is you have some measures, these o-graph methods, that give you an indication that it will or will not have the kinds of qualities you are looking for. That somehow has to be related to structure--function is a result of structure.

Some of the stuff that Brad is working on with NIR, and Raman stuff should help too--
---Yeah. Right.

I just asked Craig, could you create an antibody that would recognize a broad protein conformation like a beta sheet?

Oh definitely. Yes.

We can't move that into the short term?

To make an antibody, if we had it figured and knew what the target structure was, it's going to take 6 months really, and then another 3 months to develop something (???). But first it sounds like we to figure out what is the structure, what is the target you want the antibody to recognize, and that sounds like it has not been deduced yet. But antibodies recognize 3-d conformational structures; that's what the whole thing is about, so if there is a structure that is responsible for this function, then ultimately an antibody should be able to tell you whether or not that structure exists, and what it's concentration is.

Can you relate this to the glutenins George?

There's been a world of information relating glycosidal proteins to ???

--philosophizing--

That's why you have to go back to basic rheological measurements.

Well that's what we've got to relate to ?? too, is fundamental rheological measurements. If you think that cereal chemistry and rheology cereal chemistry is the whole of rheology, you are wrong, because industries: petroleum, plastics: all these guys have a very good understanding of how polymers work, using basic fundamental transferable units of measurement that everybody understands.

Well that's why I think the ultrasound has potential, in tandem with,

Turned tape over

…follow up on what Jim was mentioning about knowing structures. One of the ways down the road a little bit is some of the chip technology. If you can define what is to be measured, in terms of structure, then one can do these sorts of measures not just by ELISA but by a variety of different

chip technologies where you measure interactions, but again this depends on what you need to measure and that structure-function relationship.

(Can't make out a question about biochemical component)

Yes. Well what one could do is say OK, you've got 5 kinds of things you want to measure; you may want to measure soluble protein, you may want to measure 3 or 4 classes of glutenins, you may want to measure any of a variety of characteristics at the same time very rapidly; and using chips (???) you can certainly do that and quantitate them. It's just that the up front costs are very high but the machine for doing them is dropping like a rock.

Tim: Are there other technologies or derivations of technology?

Well I think we certainly would put PCR on this list.--
--Longer term?

Something about moving from blended samples to pure varieties.
PCR would be expressly for variety identification. Maybe even on a kernel by kernel basis. I think in those small but growing instances where milling the varieties or because something they preferred or don't want, the technology is ready to go now. But I think it's not common enough for both those????????

The idea that those people, say general mills for example, who may be interested in milling specific varieties, validating that or knowing the mixture of varieties, ??

Can you develop a strategy that can tell you if cultivars are included by proportion?

Certainly one way is ?????? is use 50 kernels or 100 kernels or 200 kernels, and establish the proportion from that sample.

---But that analysis is going to be pretty cumbersome and long and difficult because now you have lots of PCR reactants and primers for each of the varieties you are looking for. So I really didn't mean to say it something that couldn't be done, but if it's going to get done at a grain elevator, I don't think it's going to be anytime soon.
-Definitely a centralized lab-
-And I would also agree that all the people who must be dreaming these days, the developers of varieties, are probably relying very heavily on that technique to help them characterize their varieties. They are probably looking for unique sequences and protein spots that give their varieties the characteristics they are looking for.

I think we are just on the cusp of that in terms of market driven selection or traits that are diagnosed as ???. The one place that it will really take off is when they have markers that ??????. those are so difficult to evaluate.

Another comment I would have is, we've been talking about what could we implement, and that industries are reluctant to change and I think from my experience that observation is true in almost every industry (except information technology). I would point out however that in the case of agriculture and there was also starlink, which was a highly motivating force, that the entire US corn supply changed its way of being handled, and the entire supply went from no testing to complete testing in 1.5 yrs. So we have 2 examples of agriculture changing dramatically in a very short period of time, 1) in the case of Starlink and 2) in the case of the Brazilian soybean crop which is tested entirely now for things like GMO's. And I draw corollaries to variety testing, and GMO testing. GMO is a variety of sorts. I think that this concept of identity preservation, I recall vividly 3 years ago being in conversations where industry says this will never happen, the infrastructure is not there and it cannot be done; and in the space of 2 years that has changed dramatically; we talk a lot about IP and what to do and it sounds to me like IP is the kind of thing that could happen here, and while we are talking about commodities, it sounds to me like consistency is a very large

problem here, and that the two things that effect consistency are environment and genetics. And the genetics are best predicted by variety and class. And then someone from general mills said they got their best consistency improvement when they controlled varieties.--
--by region--
--but by region is then an issue of the environmental factors, soil light, etc. so it seems to get the most consistency quickly, and there is evidence that it can be done rapidly given the Starlink and Brazil GMO bean situation is IP; because if you do IP ;you will know your variety and you will improve consistency immediately it sounds like and then to get at the environmental effect on the genetic background there are existing o'graph techniques and we're trying to find some way to correlate rapid methods to the existing functional techniques; and it sounds like the IR techniques.

(Comments on IP, cost recovery, world competition.)

Didn't someone ask the speaker from General Mills whether or not they paid a premium on the IP material?
Yes--he did say that.
He did pay a premium and I think he was saying that the real problem there was the concept of sharing the value.
Absolutely, that's a huge problem. And another huge problem is

The other part of that dilemma is this: where do you get the greater value in doing the IP by variety by region or understanding what that functional quality is, that you're getting out of that variety by region, measuring that, and binning on that basis and worrying less about variety by region. Again ultimately that's going to have to take you back to that molecular level to really do that well, but point is ultimately I suggest we will wind up at the other end of that spectrum, once we really understand what's interacting ,,,,

and it's happening now. we had a talk by the FDA a couple of weeks ago, and they are talking about moving this thing clear back to the farmer. They're talking about a declaration. they're going to have to keep track of every load of this grain and where it's going so they can tell where this grain has been at any particular point , just like any other business, FEDEX, WalMart, whatever. That's really going to bring it around to more of this IP, They're going to know where this particular boat came from, where it's mixed, where all these things are at, at any particular point in time.

But what we found out is that there's many things they can test. We listed 60 or 70 methods yesterday. You can test for all those kinds of things. And if you had a completely unknown sample, and you didn't know where it was or anything about it, other than it's wheat and it's got a good test weight, you almost have to do a full battery of tests to get enough information to understand what it was and where it's been.
--so then when you segregate you…a whole range of rheology tests and based on that …
--But the advantage to the IP system is that while things can screw up along the way, there is no paper trail that is completely infallible. If you have an understanding of what the thing is, you identified it right from the very beginning and you control it throughout the process, the only thing you are attempting to do with your testing at that point in time is confirm what you already know, so you don't really have to do the entire battery of tests. You only have to confirm that the thing has been traveling where it's supposed to and so you have a much more limited number of analyses that's required.
--keeping in mind that there's a whole bunch of different things the customers want to look at…functional level…grade related things…weather damage, falling number and on and on. Relative to the functional quality, maybe they will stop at one test but my guess, as they grow more sophisticated, they're want to know more which takes us right where you're talking about with chip technology. And logarithms(?) …do all of that.

Tim: other discussion about technology

We haven't really answered the question which is "how do we develop". We seem to have listed them but we haven't gone beyond that yet.

--there was some discussion in the short term of how but not a real degree of depth. Good point.

Well starting with the samples that FGIS has put together, and possibly in sync with NIR scan of these things ???, at least for a small period of time a technician in the lab, and anywhere from 5 to 10 thousand which would be required for purchase of a slightly different sample presentation device for the interferometer and I think we could get those ??? in for a test run. And for a small amount of …project--
--I think that 200 samples will be good instead of that whole thing (possibly discussing if there is enough material for FTIR, Raman, etc.)

Question and discussion about ARS funding, National Programs, etc.

Where does wheat fall in the amount of money the US gets in total agriculture? Top of the list?
--I don't know, I would bet that corn and beans are probably both higher than wheat in total value, but I'm not sure.
--And are we gaining or losing in the international market? Are we gaining share or losing share?
…functionality is built in. why would they come for the product if they don't have to??????????
And listening to the fact that funding is difficult for these kinds of programs with respect to wheat, and that wheat is maybe the number 3 crop and we're losing market share, I don't know, I would think that US would put a little more--
--you would think!
--From my perspective, whenever we feel we are not getting funded sufficiently to do something, we go to our industry stakeholders, they're the ones who get to hear ??? Is that not being done here or are they not being listened to?
--they would in fact have to be the driving force on it--a better wheat initiative for example
--so how do you make that happen?
--It seem to me that all the corn and soybeans I've had experience with, the funding came from privates, they were all attempting to gain market share by introducing proprietary varieties and the only way they could maintain the value in the distribution is to make sure they could test for it along the way. And so (life science
companies spent great deals of money to develop tests, they ?? for the test development.

(comments about research funding, background about high speed grain loading infrastructure, but cannot make out the discussion)

Tim: But do we have our best thinking up here?-kind of a last check about what are the technologies we can develop in the short term. We still didn't get to a lot of the "hows"-we got bogged down in the funding-that seems to be a major issue. But it was recommended that we look at "how can we leverage the system funds?" to do the best job to get something out in the short term; and how do we strategize to over a longer term period of time to increase funding in a variety of sources. But we do need to get back to the group our best thinking in terms of what are those methodologies or technologies ??? that we talked about our key indicators of quality and so on, for gluten. Anything else that anybody has?

(Brad Seabourn gets conscripted by his friends as group spokesperson while out of the room)

NIR, ELISA and SDS emerged as having good prospects for applicability within 3 years. A glutomatic approach could be investigated also. Ultrasound appears to be a longer term approach, but the primary research group gained insights as to where it might be applied to grain analysis. Jan-Ake pointed out that French researchers have done quite a bit of work in the area of NIR calibrations for gluten functionality characteristics (so we may not need to reinvent the wheel). Phil Williams has also done quite a lot of work in the same area. Collaborations between groups such as FOSS, the CGC, Ag Canada and ARS are needed and should be encouraged and funded.

How do we from the list of identified technologies develop the most promising approaches to measure the following factors?

Factors	Technologies
Variety/Class	Imaging, SKCS, ELISA
Water Absorption	NIR, ELISA, O'Graphs
Kernel Conformity (Geometry)	not assigned
Starch Quality	not assigned
Milling Yield	SKCS, Imaging
Defects (insects, mycotoxins, pesticides)	not assigned

The discussion transcript, moderated by Ron Bicsak is presented here:

…Yeah but that was for Canada and there are a lot fewer varieties.

Depends on what level of identification you want.

How about the distribution?
Hmm?
How about the distribution and ratios?
You can still put thresholds and stuff. You know, combinations: proteins 1,4 and 5 put you in one group; the stuff that Craig had yesterday that was DNA fingerprint broke it down into about 5 groups. If you can get DNA to 5 groups you should be able to get protein to 5 groups.

Something about selection in Canada
So you think that in US wheat we suddenly have a whole new group of proteins?
No but their wheat is more uniform within classes. Canadian wheat because of kernel visual distinguishability is more related within classes genetically than is US or Aussie wheat. Wrigley did gliadins on HPLC in the mid 80's and a lot of sophisticated computer pattern matching because there are various groups depending on which breeding program they came from which went into say Prime hard and lots; of within a breeding group they were backcrossing with a recurrent parent that gave them a group that (interrupted)

OK then so is it more than 100?
I don't know.
The technology that Luminex has devised, I don't know if any of you have seen it, it's a multi-analyte ELISA, you can 100 different proteins into the system, ?? flow through and do it in a matter of minutes. It's being used by Pioneer to segregate varieties of corn. It's a technology that can be used.

You don't need that many. We have 60 to 70 proteins (interrupted)
Well you don't have to use than…
But you may have only 5 to 10 very ?? varieties ?? more than 2 variations.
You don't have to use 100 in it but the system will cope with up to 100. So there's a technology that you can consider.

That sounds like maybe number one. The imaging and SKCS (interrupted)
That's goes with classification
That's right
You wanted focus on varietal.
I don't see how SKCS is going to classify
It's ELISA based technology and Luminex does it by machine
The problem with ELISA is cross reactivity. It's very hard to get rid of cross reactivity because of the structure of the gliadins and the major ones used are so similar, probably 90-95% similar

sequence in all the proteins. So there's tons of cross; the Australians developed antibodies that can ?? across that type of protein, so that's probably not technique. You just can't get all the (interrupted)
So ELISA shouldn't be on the list
Well techniques keep changing so ELISA keeps getting better. The more you start looking at different proteins like water soluble proteins or enzymes.

In the discussion we had yesterday as well we were looking at things to be able to test by ?? I mean variety doesn't give us premium price or.

We can pick out most American varieties looking on electrophoresis because we know there are one or two bands which are distinctive for American varieties compared to Canadian. We just look for one band. On HPLC and then we know it's probably an American variety. You're looking at a 2 step procedure.

Did you say by electrophoresis or capillary electrophoresis?
Well for that we use HPLC because it's quite easy but you ?can use? electrophoresis. There are distinctive bands. ?? breeding program uses similar genetic material and certain proteins keep coming through on the genetic crosses. Collect a few of those you've immediately sort of grouped it. And you can say this may be or may not be of this type and then you can go to a second step after that, which seems a very fast selection for an initial step and then you can test??

…like you're doing when you use high molecular weight glutenins 6+8… even the environment can change the ratios

it doesn't change the presence or absence
the gliadins are relatively ?? that's why they've always been used, ?because? of environmental change compared to some of the others, so it sounds to me like there's a potential technology in the Luminex technology using ELISA and it's a matter of getting any development work that would have to go on in 3 years and getting antibodies less cross reactive and deciding how many proteins we need, whether it's 5 or 50.

I need to make a caution here and it's totally unscientific but I think it's realistic. If we're talking about excluded varieties versus premium varieties, it will cause a political battle which will not be resolved in 3 years in the U.S. And it will have nothing to do with science.

Ron: so basically we have some ideas on getting variety but when we go to class, is that where we have problems, or just technology for both to identify both variety and class?

The only question I have is that I don't know a lot of this but we do have some problem visually identifying hard white and soft white. If you can't visually identify how can you do anything by imaging and how big a problem is that? I'm not totally sure how big a problem that is, but then if that's a focus of our group, then I would think that we wouldn't really know ??? back in variety.

If you've got variety then you've got class! One is part of the other one.

In terms of hard white versus soft white, you could run it through a very small mill and listen to the sound. If it doesn't make a lot of sound it's soft. (laughing) and you also have the starch protein that causes hardness so that should be a very simple test on ELISA.

The hard/soft classification, you can already do it by listening to the crushing between your teeth but you can use the SKCS…

Yea but the reality is that you're going to see blends of hard and soft.
Yeah particularly in the hard wheat region
And nobody uses SKCS, essentially, it's an expensive piece of equipment; it's essentially not used in inspections.

What Tim says, I mean compared to the price of putting a little microphone and running a little grinding roll and running 20 seeds through it to see if it was hard or soft, and this is a simple…
--except like you said, you can't do mixtures; but then if you're willing to…
--if you can't afford to tell hard from soft…
--if it plugs up the mill then it's soft…
--spoken like a miller

But coming back to your note, we're really talking protein, not ??? I think we sort of…and I think maybe we talked ?? but we're talking protein, and if you develop the ELISA, the antibodies, then the Luminex is an automated delivery, and gives you a better look at your potential for a strip test delivery system, which maybe one or two varieties are the important ones, but you've got the basis of the technology if you've got the antibodies and your delivery system becomes a question of the location where you're going to use it. And how much development needs to be involved and who needs to be involved. If you're going to have a strip test then you've got to get a strip test manufacturer involved…
--where is he by the way?
--he's in the other room.
I would agree with the comment on the ELISA technology and cross reactivity. It's always going to be an issue. It happens in mycotoxins and that's a simple situation; can be big challenges; you'll often see higher levels than what's really there when you're going ?plate? chromatography; and it's because there's cross reactivity. And that's a very simple case, where the fungi are producing ?? so there's a challenge and it's not like we're hitting a homerun with ELISA.

We're using ion exchange chips where you put a sample on there and you actually run an ion exchange for about 30 to 40 seconds so you can get separation on a chip. If you run into cross reactivity problems, the solution may be a chip at first, then run the ELISA.

We should put that up there. It's ?? separate technology or should that be…
I look at chips like something to (can't get comment—sounds like something about ??development)
--that's important. We had chromatography in the front of the room…
--maybe it should be sample preparation or something
--cause you want it fast
--not just your chips, but there's little sample preps that we do for DNA cleanup that are tubes ?that go in the centrifuge?
--I can't think of any cleanup any of the ?? stuff because it has to be a ?? color, it has to be simple…
--yeah you may want to leave sample prep as one thing but the chromatography is really the actual technical term it's been defined as. You're talking about more than just sample prep. You're talking about doing the actual separation.
--no the separation occurs right on the chip. All you do is put it on one end and the color comes out on the other.

Are we talking about technologies here that are just restricted to the assumption that you have a single variety that you are trying to determine? Because I see things where we've got 7 or 8 varieties in a sample where there's a complaint.

I think this technology could look at all of those varieties.
--you could resolve the challenges
--you're contamination in your sample prep is not going to just come from other proteins, you need a fast way of cleaning up the samples for when you go into the ELISA technology, you don't get quite your normal ????????? that could prevent you from seeing the ?????????. So some type of automated prep, which might just be in the collection tube.

The nice thing about that is very small samples so that extraction is very fast.

It certainly would be very important to (can't hear rest of comment but may pertain to capillary electrophoresis)

I think we have the expert in the other room on ?? We may have experts here—I'm certainly not but I don't think we can overlook capillary electrophoresis. Some of that work has been done, especially pattern matching. A lot of that work has been done, though not for the US.

Are you talking for the elevator? Or where?
-- well, it does do ?? now. CE is a very, it's relatively inexpensive if you think about what's involved. The hardest thing is getting it on the column, but it's just a set of buffers and some electricity and there's nothing to it. And it's fast.
-- our elevator agents have trouble turning on moisture meters (laughter)
-- yeah so this may not be it after all
-and they are just as likely to kick it
++ and that's why I like the ELISA
** but we shouldn't exclude DNA. I wouldn't cross DNA out –for the future, because it we have the whole …
what about mixtures?
-- if you are doing it at the elevator, you'd kind of think that the growers haven't planted 7 varieties in his field.
-- no but if you're at a terminal elevator or where they are loading a boat…
++ yeah but I ??? come up yesterday and despite the fact that it seems that it would be very nice to have the same testing procedure right through the supply chain, is they can have different needs at different levels of the supply chain and should address it that way.
== I agree totally
++ yeah, until you start looking at the ?seed? and start running identification on 300 individual kernels, so you can do the stats…
-- well you know I said 7 (varieties) but one of those 7 is 25% quote unknown. Even if it's our own varieties.
++ Yeah but you can end up with an unknown classification for variety ID; you might just come across a wildcard that you've never seen before that you identify as such.

?? The innovation is the fact that you can separate soft and hard reds and what not. And to have it a ?? is making a class rather than looking at variety is step one. Then take it to step 2—variety. Variety belongs in a class. Might be a short ?? in 3 years.

But aren't they telling us class is not good enough?
Class doesn't get it all.
It doesn't get the quality

Well for example, you know if it's variety X it's got partial waxy starch and that it's red seed coated that it's…
You just said the key word there—variety. I think that's why it's up there.
You don't even have to identify; I mean I know; I've looked at the difficulty of trying to look at hard reds versus very hard vitreous hard whites from Australia to visually identify them using ?back? lights. ?? illumination, sideways, imaging, all sorts of, and it's very difficult, but if you know that it's got; that it's variety X and you've identified it and it has 3 red genes for red seed coat then it's red. And it if it's hard it's got the genes for hardness and you know those genetic genes don't change a lot.

Well a political problem again here in the US is that we've got several different kinds of hard wheat that could conceivably be coming in to the same collection facility. Hard red spring and hard red winter. The SKCS will tell us whether its soft or hard but I'm not sure you can tell a spring from a winter. I guess what I'm saying is I'm not a fan of our classing system by any means but on the other hand it is a political reality. And I think politically we've got to keep those classes separate.

That is, for instance this kind of automated ELISA, and there are some technical issues to overcome; we've advocated, we'd find people who are experts in making antibodies and we'd find people who are experts in automated design of chips to clean up the sample; and then we'll know that Jagger is a hard red winter and MacNeil is a hard red spring and once I know that, I don't have to ? end use?, they won't ??

== exactly, but if we are talking about something down and dirty at the elevator, we still have to be able to distinguish between these politically important classes

RON: so are these the type of technologies that we want to go forward with or want to recommend that we go forward with to determine variety ??
** I vote yes

RON: Is there anything else before we move on to the next one?

Well what does it take to get that to the marketplace? That's the question that I want to know.

RON: so we want to, these are the things we want to look at, if this is already in place then there's not an awful lot to do other than get a hold of this into some laboratories, and running samples thru it to determine how well it works; in the other area, the ELISA area, we are going to have to get a hold of manufacturers or grain companies or whatever who want to put forth some time and effort to see whether this methodology works.

What is the Luminex again?
** It's ELISA based.
--So should it go under separate technology?
** No. ELISA is your technology. The delivery systems are Luminex, strip tests, plates, however else you want to deliver the ELISA technology.

And what is it being used for currently? Medical or…
** Mostly medical, but Pioneer is using it to separate varieties of corn; the data's not all complete; they've got ?? separations. The European Union Joint Research Council is using it to separate modified protein. they are finally recognizing that PCR takes too long.

Question about how it deals with mixtures.

Well yes, you can separate 100 different antibodies in the same ?microtube?

Well I think the question is not whether you can test a mixture but whether you can determine say a degree of admixture. Let's take a simple case, of 2 varieties. What if it's 5% and what if it's 10%

But that's part of your ELISA technology. I want to say your ELISA sensitivity, I'm not sure that's the right word but how much it actually picked up. It's the same type of technology that's being used today to design ELISA tests for genetic traits. For modified genetic traits. You use your affinity level to determine what level or threshold…you could do qualitative or quantitative, you set your affinity to do that.

That makes me a little uncomfortable. I don't know if you've got the unique proteins that allow you to detect…
Yeah, that's what I'm getting at.
With the genetically modified gene…
Yeah I think it ought to be investigated, absolutely.
But you have bands or groups of proteins. You don't necessarily get it on one, if you might have 5 or 6 of the proteins showing up, you know it has to be in one group or another.

So who develops antibodies-for wheat? Has anyone done it for cereals?

The Australians have done some work on some of the antibodies and cross reactivity's. John Skerritt (?) has done quite a few of the high molecular weight glutenins.

RON: so the thing that has to be, if it is to work, we have to identify these proteins.

The USDA lab at Albany, CA has one of the best labs in that area. Anne Blechl.
The reason why I think the characterization of proteins is so important, it seems to me like CE and powerful techniques, not saying all the way down to the farm level necessarily at this point but for the future, but CE and those technologies have an advantage in a sense, that they go right back to the traits. Forget the variety; eventually if you have expressed proteins, you know what you're going to have. If you have strong gluten or weak gluten, gluten you know the whole thing. So CE addresses both issues at the same time.

Turned tape

The problem with proteins though is that you may be able to tell varieties by ??? . For instance, if you know a variety has 5+10 high molecular weight glutenins, you can make a prima facie case that is has strong gluten. You can make that case but it's by no means set in concrete; because then you've got the other two: ?something about water solubles and high molecular weight glutenins? , then you've got to factor in low molecular weight glutenins, then you've got to factor in the gliadins then you've got to factor in ??; you cannot make a decision on, you can make a prediction using the protein pattern to say 'what's the functionality?' but when the companies of the world are actually adding water to the flour and start mixing it, then you've got a lot of other factors that come in: the ratio part, …

Something about biochemical characterization
But everything in that seed is everything you need to make it what it's going to be.
No it's not. You have the some of the physical things that happen during milling,
Something about Particle size, hydration rate, you know there's you heated it up too much during milling.
Part of the solution is put some money in and send the people that you want to do it to medical conferences for the next year. You may find a solution. Because they are so far ahead on this stuff and that's where they are going. That's where all their money's going.

George Lookhart was borrowed (from the other group) to discuss CE with this group.

Can someone bring George up to speed on the discussion. We're talking about variety identification and how we do that.

RON: Tim, since you want to, I mean it's your…

--In all the reading I've been doing, and you may have enlightened me a little in terms of the technique; if you can actually take the proteins we think play a big role in this whole thing, and we can we characterize them quickly with something like CE, which is a very powerful technique; and not only is it powerful in terms of variety identification but eventually it could go back to the traits, or maybe it doesn't go all the way back to the traits…
++..certainly couldn't deny that there's a prima facie case that you can make a prediction and the more you know about each additional protein and it's effect in that whole matrix thing, the closer we'll get.
--do you do CE now in your lab George
**yes it's quite a simple technique; you take some electricity and a couple of buffers, a power supply and a capillary-that's a little expensive.
##what is the preparation time?
**preparation time is 5-15 minutes for the first sample then everything else after that runs in 3 to 4 minutes a sample. After you get the first preparation done, you can run 20 to 30 preps in the same 15 minutes; so you have a sample coming off every 4 to 5 minutes after that.

++you can ?? 4 or 5 minutes but for each individual sample it takes 15 to 20 minutes; so you can start a sample every 3 to 5 minutes –Yes—so there's an overlap with individual…
**yes; if you want to do CE with like our IBA buffer, diacetic acid buffer, you can do analysis in 4 minutes if you want to use that buffer, you have to extract out the albumins and globulins first and then do the prolamines and gliadins. You can do where you just extract the prolamines and gliadins first, which is a very simple 2 minute extraction but then you have to use a different buffer, you have to use a phosphate buffer, because if you use IBA, then the albumins and globulins tend to plug up your capillary; so there's sort of a trade-off with different buffers; but that takes about 10 minutes so by the time you get thru it, and it kind of a wash for one sample but if you're doing multiple samples it's best to do the albumin and globulin removal, then your gliadin, and then every 4 or 5 minutes you've got another sample coming off.

--The power that I see in that is OK you've pulled out these grains and you've learned two things really important to not just variety but perhaps back to the trait; OK you've pulled out those things and now you're focusing on them, rather than trying to, lets pick NIR or something like that, that has everything in there-well you may have a good chance too but probably not.
^^that sounds like a prime candidate for being a strong possibility for both of our groups
%%you've got to be careful getting into a 3 minute technique or 1 big technique. I don't think there's anything in between. What you either want to do is centralized lab testing or 3 minute on-site testing. If you're looking at a centralized lab, there are probably 10 or 15 techniques that might work; and it's not time dependent, it's how many samples can you put thru; whether it takes 2 hours for the first sample to come thru or 8 minutes, it doesn't matter. If you can get 500 done with one technique and 300 with another, even though it takes 5 to 10 hours.
--but you have to consider the power of the technique too; if it's a really powerful technique then you might be willing to wait 5 more minutes.
%%There's ?? electrophoresis, there's polyacrylamide gel electrophoresis, and to some extent, and perhaps mass spec and a few others. If you work on one it will go ahead of the others; but I think all of them work.
--and then you've got to look at the throughput you want;
**and you'd be looking at different kinds of proteins and separations. If you are looking at free zone capillary electrophoresis or reverse phase you're looking at better separation and more information characterization of these individual proteins. If you want to look at something like size exclusion or chromatography or electrophoresis CE-ME or if you want to look at gels for like SDS, you are going to look at size based separations. You are going to get groups of sizes; if you really think you're really interested in what are these very high molecular weight things, or if you want to look at ?? something; then these hyphenated things with a mass spec on the end of a CE or a mass spec on the end of an LC; then you're going to be looking at all these groups with all the information, more information probably than what you want, but at least I think with mass spec on those you're going to get the complete structure on that protein if you want. And coming off as fast as they're coming off with the new computers and data sets today.

--and you said you've actually been successful in some of the mixtures too, right? Like you'd see at export terminals

**it's very difficult to do mixtures by any of these techniques. But what I do, there's 2 ways to go: one is the brute force where you do single kernels the other way, which I normally do is send them back to the guys at the board of appeals and review and let those guys who look at them every day do the segregation, send them back to me and then I analyze each of those to identify them. That's the easy way.

%% what happens if you don't have a board of appeals and review to segregate out the kernels, do you just throw them in a CE?
** you've got total garbage.
-- how much work has been done in terms of, like has been done in NIR where they've got partial least squares fitting, principal component analysis…
** for variety identification?
-- discriminate analysis. Yeah

** it hasn't been done. It should be done. Well people have looked at it but no, you wouldn't be able to tell. You've got 50 peaks; most of them are the same for these varieties; there are differences in intensity, maybe slight differences in position. And so if you've got even more; if you've got more than 2 varieties, your chances of being able to pull them apart from that mix, without separating individuals are almost nothing. And if the other one is less than 10%, you will not see that second variety in with that pattern whether it's HPLC or CE.

@@ But don't we need a ??, and we don't have time, so in that case would ELISA fit here?

++ I have a horrible bias in that situation, which is variety declaration and you back it up. It's simple it cost effective and the devil works because the whole country uses it.
** And then you can centralize your testing facility.
++ and if you need to you can go to a kernel by kernel if you have a litigation.
-- you talked about controlling the varieties…
++?? declaration. I know it does put the onus on the grower, but if you are selling into a quality market, you better start thinking about quality.
** I think the declaration is going to come; because we just had a seminar 2 weeks ago; we had people from food safety that talked to our local AACC section. They talked about how they are going to force farmers to keep track of everything from where they grow it all the way through. They're going to put the pressure all the way back so they can control everything, they know where every sample came from. FDA. Excuse me. That's scary.
== but that's happening everywhere. If they can do it why can't FGIS do it?
** you think WalMart doesn't know where the leather coat comes from?
+* the amazing thing is there are logistics systems in every other industry, but for some reason US commodities are saying "oh we can't do it" but everyone else is doing it.
RON: OK I think we've identified the areas we want to look at, we've identified what we have to do to get there, and some future possibilities, so unless there is strong opposition we're going to move on.

I think Andrews point should go on there, which is varietal declaration.
++ basically what George just said is that having any of this technology at a port is of zero value because we already know they are all mixed.

RON: They have listed NIR, ELISA,
-- The o'graphs
== and again they came off of the spread sheet. And we realize water absorption is a function of many things.
RON: So where do we want to start? Which technologies do we want to list here besides the o'graphs?
++ I guess ?? because NIR can do it to some extent, but if you want it very precise NIR you won't get it
// how precisely can you get?
++ well truthfully when you do an NIR, your ability to predict water absorption is ?? by the linkage between protein content and starch damage; so if you remove those components statistically, you'd still have some ability with the NIR of picking up related to water absorption

can you do it on whole grain?
Uh, I think so, I'm not quite sure (something about excluding starch damage)
++ you better do it on your milled products because there are mechanical implications.
you can do it on wheat, it's just not as strong of a relation but it also depends on the finished product.

How about sedimentation? solvent sedimentation? our work is only starting. ?Some of the solvents absorbs and after a single centrifugation we measure the height? (*Mik's comment)*

** Problem with solvent retention is it takes a little time to ?? and you're looking at chemistry now because of diffusion limited responses to polymers to hydration..

Put that on the declaration…
Yeah guaranteed to absorb water

++ Looking at the potential involving water absorption, perhaps we'll say this wheat is not giving the same absorption as that wheat. So look at the potential of the grain to give water absorption. There are some milling companies we work with which are doing it routinely. And it's working well enough to; what they do is get samples from ?*declarees or referees*- (*Steven's comment)*-you were speaking here ? around the country and then they map the country for certain traits; and one of them is water absorption.

-- is that one of the keys words though is ? (*can't get the word—Andrew's question)* ? rather than..
** Yes absolutely
++ But you're looking at a low correlation; you might find an r squared value of point 6 or point 5; but it's certainly strong enough to give that ?? added value; so you have to say' what value is that to the market?'; and I don't think you're going to want to spend millions of dollars on something that you're not going to get ??

// maybe r squared for water absorption on protein ?? so why not measure protein and hardness by nir and water absorption by ??

++ the problem is that when you do water absorption by farinograph, you've milled it and you've added mechanical stress, etc. so how do you calibrate your nir against that? so there's a lot of introduced noise.

We focused about sophisticating the customer whether it's domestic north American or export customers and they can look or be trained to look at protein and hardness as predicted by nir for which we already have technologies and make their own decisions based on ; maybe we're trying to predict one thing too many because it changes so much. Australian wheat farinograoh absorption and no time bake abs

++ I can't imagine having an absolute numbers for water abs being a contract spec
-- but it's important to know that this particular shipment has a much higher water abs
++ yes I think that's possible
-- low medium high
but also if you know the variety shouldn't you have an intelligent guess on water absorption?
++ possibly
// a lot of it is determined by the milling?
++ oh yeah. It's the design of the mill actually-the flow diagram

RON: so are there any other possibilities other than looking to use NIR for protein and hardness? is that reliable technology that we've come up with or is there anything else?

I would put falling number because it is ? different absorptions…
**that's a separate test we would do and factor that into the whole buying contract and maybe that's already embedded in the testing that we do.
-- I would ask the people who know about NIR would it help the correlation for it to be milled—presenting it as a ground…
++ well, if you're trying to get the hardness, yes, because you are really not going to do much for hardness determination by doing whole grain NIR analysis. You're asking too much.
// one of the systems we delivered to GMRL this week was is a single kernel ??. we have a new one that does single kernel nir.
@@ I would caution one thing here as a practical miller rather than a lab miller. Things are a little bit different with respect to hardness and water absorption. Harder doesn't necessarily mean you're going to get more water absorption in a commercial mill. A lot depends on how much wheat you're putting thru that mill.

Thinking about NIR as it exists today, as we have them out in the field, is a water absorption equation that gives us low medium and high, as we define low medium and high, a broad range, 5% increments, 10% increments, is that??

Today you can do it because you can measure protein. your field instruments…
++ I think protein and water absorption is the best correlation in all of the export cargo data. Just those two by themselves.
-- the other ?? using in Europe is ?? wet gluten. And it certainly correlates ?? you're looking at proteins and so forth.

New tape.

… protein and hardness. The group is not saying it would be an NIR to ??? water absorption. I think that a point 5 to point 6 r squared on that; we went out there in the official system…I've seen a point 5 or point 6 correlation and it looks like a scatter plot.

I've got the data I think on whole grain kernel spectra… and I've got water abs.

If we're looking at the overall sense, look at the consistograph. You've got a ??? direct to the farinograph… water absorption.

Most of these systems are for flour samples, they don't have high bran contents.

I think it's worth just having in mind that there's the possibility of some development work going on there where you do grind them and run them through a sieve and sieve out the bran…

Question about mixing times

There's mixing technology that Ken talked about, some kind of centrifugal meter they can mix things in ?? conditions, maybe later on that kind of development might end up …

I think it's important to do an experiment with sedimentation of some sort. I'm talking substituting ???…

I've got experience of ??… scaled down wet chemistry types of tests into receival stations. And just really basic and ?? … thinking about buffers and thinking about delivering x amount of solution, and Ken saying that they had trouble getting people to turn the moisture meter on. We had to deliver 25 ml of water to a bowl and then add in 4 gms of wheat meal. .That was a nightmare. And in different iterations of that instrumentation we thought about whether or not we'd look at the native starch without the alpha amylase, are we going to add the RVA??, do we analyze ?? at one place we were going to add silver nitrate until we realized it was poisonous, so anytime I hear about doing anything to do with grinding, extraction, buffers, you have to think about the delivery system of the buffers, whether you send them out premixed or as concentrates, or rely on someone to add the concentrate to a known volume. Washing a cup or tube for RVA or FN is a big issue if you get a little dried piece of material that affects the test. Some receival stations had water problems. When you think about any type of wet chemistry you have to think about those kinds of things.

You do have kits like mycotoxin kits out there where that stuff has to be done.

Mycotoxins are a big issue.. there's a penalty for not doing that correctly.

Our experience in Canada would totally align with that. For the RVA, to try and get people to weigh accurately, they may weigh accurately but then they'd drop half the sample on the way over from the balance to the instrument and just sort of brush it back and call it all right.

Same issue on the falling number. How many times do you scrape the plunger? shaking, etc.

Instruments have to be user friendly. It comes back to the big argument. It's the total system and whether you can centralize things with trained individuals in a laboratory environment where you are actually looking at point of delivery testing.

Pat: Remember we are looking at how are we going to deliver information to the import customer and that information isn't going to come directly from a country elevator, that's going to come from an export boat being loaded, so in light of our conversations this morning, how much are we getting at marrying factors and technologies which start begin their useful life at an export facility while we are inspecting grain and then trying to back that up into the country?

How long does it take rail cars to get to the port or your trucks? 2 days? So you've got 1day?

If you want to test rail cars if you're interested in export??

Pat: But what we test is what's getting pulled from the bins in the house and going to the shipping bins to load onto the vessel, which is where we collect the sample.

Now I'm confused about the mandate. Because the mandate was reliable, robust, etc that can be done at the driveway

You're changing the overall parameters. *(GIPSA hoped to focus the discussion primarily on testing at export, not the farm gate.)*

Andrew described his inspection room

I think …is right, we're putting things in boxes again where one of the first issues is that multiple values would be very desirable, so that now we have milling yield and water abs and protein content

RON: Moving on to next topic

Talk about kernel conformity now.

Discussion of morphology vs. geometry for kernels. Is it morphology or geometry? What's the dif?

Morphology concerns long vs. short rachis's, apical head vs. none. Etc. Morphology is the characteristics of the grain. Geometry is diff.

So are we talking about morphology as a biological characteristic or talking about the size?

I would like to go with geometry. The shape and size. geometry covers shape and size.

Geometry relates to milling yield. we have that on the board as well. Ultimately they are very closely related to each other. Size and shape relate to milling—that's why I would want it. The main factor is cleanout. If you have large and small kernels and you have a 2 ml screen like the Europeans, you lose all the small stuff; all the HRW comes out.

So the first reason you have size is clean out. And the second is milling?
Yeah milling and there's two different aspects.
You need uniform kernels and theoretically bigger kernels have more endosperm., although I'm prepared to argue with that.

There is a relationship between kernel size and milling yield

So are we going to use the word geometry? Yea. Ok now that we have the word, what technologies do we want to use to determine this?

I think that the SKCS does at least part of the job, we've already talked about imaging, and if you could marry those technologies you'd be in wonderful shape.

There's some research being done.

Some comments about sieving tests in elevators and doing things the same way every time.

(Steven) Actually in our program that's one of the things in our breeding program we trying to reproduce is using imaging to predict sieving outcome. So then you can eliminate some of that person to person variation. And it's a lot quicker.

Is there an imaging test ready to go into an elevator? No not now but there could be one in the timeframe.

Something about adding imaging to SKCS?? To add imaging to SKCS ???

(Henry) I think one of the problems is, and I don't follow the literature, the technology is pretty much there but the software is not. What do you do with the numbers that you get?

What about ?????????? we've got it up there with??????????

Yea if we're looking at delivery points, we're rolling out imaging systems for the elevator and producer level, and looking at $15K Canadian, which is like $10K U.S. and primaries are gagging at that sort of price. So if you are looking at 35-40 ?? you're not going to find it, long term maybe yes, but not ? and the handling companies are all kind of bleeding and saying well why one for a central facility? Well if you do that again it comes back to the just the whole…

Yea but I think you're looking at volume equipment.

So what are you putting in?
We're putting in imaging. A system something that's available? Well not for cereals as yet. But yes it is commercially available. And it's the camera? It's a flatbed scanner. We're working on cereal application software. That's on much longer term.

With that comes some work on singulating or ordering seeds. For cereals we had to have a singulation system. But it's really quick and we lay down basically a sheet of seeds really quickly. And we're dealing with the touching seeds and most of it is that if you can get a low percentage of touching then you can ignore a few. If you're looking at say 1% of the samples, who cares.

Comments about robustness of equipment.

Are we talking monochromatic or color imaging? Trying to tie color in brings up RGB calibrations which makes it more difficult and makes it more important that dust and stuff doesn't get in the way.

(Steven) We've spent 4 person years just working on a color calibration; and now we've got that down literally that you can put a calibration chart on, push GO, and the system will self analyze. Henry was asking yesterday, "what resolution?" And the resolution is just enough. Depends on the question you're asking; you don't want to get more information than you need to make the decision; it costs you time; computing is dirt cheap these days; ?? comment from Anne.

(Steven) It's a whole bunch but I think one of the advantages of a lot of these technologies that we've looked at, even with NIR, is that we're looking at dealing with single samples as opposed to how can we implement more to monitor a lot more samples. Because wherever we're dealing in the business, whether it's a farmer dumping a truck—it's 50 grams of grain representing a truck; or a kilo of grain, representing 8 tons out of a rail car. So a lot of advantages of the system, maybe they don't have the accuracy and precision but if we can use them to do more continuous

monitoring; I know one of the results we just saw very recently, using rva and it was 80 samples from a single railcar, it was scary.

Did you follow the GIPSA sampling protocol? *laughter*

I have a question, has anyone done anything on the 3 dimensional shape; does the plumpness of the grain have any value? Yes it does and there are various ways to get at it, but not easily.
So if you just ran a and measured how high each one was like little mountains, like a topographical map—yea we've done that- some comment about white light and other sources—

We could put a wheat kernel on a little pedestal and shoot a laser at it…
Well that's what I had in mind actually.

Put milling yield on the previous sheet before we move on. Kernels and milling yield are the same thing. We should throw NIR in there also. Milling yield is a combo of the physical characteristics and the internal structural characteristics—both aspects are needed…

I think maybe we are talking about actually, you know we're going to get information from NIR about protein and potentially hardness but we match with imaging data to come up with the potentially milling yield rather than it being on its own.

Well I had visualized that you'd say that they're saying that this is the theoretical percentage of endosperm in this wheat kernel, and now you millers get whatever you want out of that.

OK, Starch Quality

There's an ELISA test to look at one aspect of starch quality.
Give me a little primer, very basic, what are we talking about.
The main difference we are interested in, in the current stream, in terms of different starch properties, is the presence or absence of one of the copies of the granule bound starch synthase genes that make amylose. The partial waxy. So the most common one when they mention one is on chromosome 4a and these are enzymes so they are very specific proteins, you have 3 copies that come very close together on an electrophoretic gel; one or two of them is missing you can make a case that the starch will swell more when you cook it.

Well to what extent are we looking at starch chemistry vs. starch morphology when we talk about starch quality?
Well I think again the word quality is going to get us in trouble, so let's be clear about what we're really talking about, which is starch identification or characterization.

…granule size ratios

one aspect of starch character that is very important is amylose/amylopectin ratio, which kind of a precursor to this partial waxy concept. And there are the ELISA based techniques, the DNA techniques, the electrophoresis, flour swelling volume, the rva,

As far as granule size, I don't know if there's anything which measures quickly, maybe an nir technique?
We do starch profiles using imaging. It can be done pretty quickly. What about a microtrac type size determination? We've tried it and had some success but not great success. Because of sample prep or??
Well if you go to some of those techniques, you have to get pure starch out, whereas if you use imaging you can do it on a grind. It depends on your final outcome. If you really want a detailed analysis you need purified starch-that's the way to go; but if you're trying to come up with a, we've done it with our barley group to screen hundreds of lines; if you haven't got time, you just want to get thru and pump samples thru, need a quick and dirty technique;

I've got a feeling that in my world the interest in partial waxy starch at the moment, maybe there will be more interest later, but in starch it's the partial waxy character; that's one more thing to measure; I mean I don't know anyone that I've ever spoken to in the industry that said the want more A granules, or we want more B granules, so I don't think that granule size currently is currently within the parameters that people want to deal with. I think it's one piece of information too many. Yea there's no knowledge of particle size properties and their impact on processing. There's some but now much. Not enough to include in spec. The key thing that we can manipulate right now are those 3 genes in various combinations, and that's what we ought to be chasing if we want to chase it at all in the marketing chain. I don't even know at all, if we go back .

What do you guys breed for? Full waxy or partial waxy's? some of both, Nebraska and North Dakota are breeding for waxy's. they're an ingredient…will get segregated in the supply stream. I know Craig Morris' group has several single null's and there are double nulls, but at the moment the most common one is the null variety the wx1 B null allele, which is the most common in commercial germplasm in the world. If you know the variety you already know it.

Well but wait a minute. ?? this is an ELISA, there's already one of those monoclonal's …you can go back and use in variety separation. So some of these do link together. …because there's some information?? here that belongs back on the first page.

And this is a non storage protein that's fairly stable across environments that we could add to the gliadin for instance to make one more, in terms of doing a patent (or pattern) match or a number of things on a strip that would say, OK we've narrowed it down because we know that only certain standard varieties are commercially available on all null 4A so it has to be one of the other 2. So we've already made a selection based on that. I would say that this is part of varietal identification.

Well I'm very much a layman here and you may have already explained it but when I look it from a practical standpoint, one of the complaints I hear overseas, is that wheats with very good FN and very low viscosity. Is the type of starch characterization something that you're ….

Falling number doesn't say anything about starch characterization.

Well see that's my question. Are we talking about starch characteristics when we've got this problem or are we talking about amylase.

If you've got high amylase content in a starch granule it won't swell as much and so you get a lower peak viscosity. It has nothing to do with amylase action on the starch.

But I think another point you raised there Andrew, is that again depending on how long you have to do the test, that you can use a single technique at multiple different layers to do branch decision making as opposed to hit it once and come up with all the possibilities. And again, that may not fit the three to five minute window, it may for a test but again you may have to get to three or four layers to again get to the level of information you want.

OK we're going to have to go on to the next one which is defects and we had some discussion there on what we wanted to define or what we wanted to work on. Whether we wanted to limit this to do insects or mycotoxins or pesticides.

There's two categories: one that you can detect visually and one that you can't detect visually.

On insects? On insects, mold damage you name it, all kinds of defects.

…?? detection by ELISA.

Yea, I heard that and it rang all kinds of bells. About 5 or 6 years ago we actually did put that kit in and we prepared, calibrated it, ran it for fragments the problem that dawned on me yesterday is that on that particular kind of test, you get a number, let's say it's five, and on the test the calibration

tells you it's five, but what really brought it to five is one big fat cockroach. And that's one fragment. And there might be ten beetle legs that give you the same number, but that's ten fragments. And so the test is reproducible, robust and all those things, but it doesn't match any usage and abusage of insect fragments. So it doesn't really directly correlate.

We first have to decide what we are going to talk about, are we going to talk about all three, are we going to eliminate the…

Someone suggested that it was physical/chemical earlier, going way back.

Yea I mean, you took the mycotoxins and pesticides other safety issues. And the significance and importance of those is tremendously greater than the physical damage: shriveled grains, fungal discoloration, or anything else, to me they are totally different …

I would also suggest just from what I am hearing, talking about insects, there's a lot of promising technology being worked on right now, so we don't really need to give that a push because people are already interested in it.

Well I agree there's a lot of interesting work going on in detection of insects but I think to take it from the lab into the commercial environment it really needs a big kick up the pants. That's a huge bridge to cross. Lots of work going on soft ?? X-ray detection of insects and it's imaging, that's very promising. And the acoustic was brought up with the…. and that's an imaging technology.

Discussion of bioluminescence.

Do we want to put down that bioluminescence has a qualitative determination?

It's very rapid, it's ….

I think that's an interesting strategy too because toxin detection can do detective work. You may know if you re in a fusarium area…vomitoxin, you may know other potential other ones, it might be that you have a pretty good idea of what pesticides generally you possibly are going to be used that may have residues that you're looking to detect, that may not be difficult but to have a qualitative indication …..

Do we have to put image analysis in there?
I think we should. To say that image analysis just belongs with kernel geometry, the reason we have geometry up there is we were thinking in terms of potential milling yield, which is different than why we're considering it for defects. It has to be up there too.

Question about correlation, mycotoxin damage, imaging,

I don't think imaging in terms of predicting mycotoxins, but you've got all sorts of insect damage, you've got things like fungal discoloration, and all that, which you can probably do and relate thru to end product quality.

What about broken kernels aren't they defects?
Technically no but yes they are defects.

Imaging can help detect fungal damage, and it's very respectable…is there a way of illuminating the grain not to detect the physical damage but to see the presence of the mycotoxins? That you can illuminate it with fluorescent light or some other way that would make imaging do two jobs at once?

It would do six jobs at once. If you take and capture that image, depending on what wavelength you use to illuminate, you get that information back.

You're not going to successfully do that with a mycotoxin, toxin, pesticide area because you're looking at too low of a level. You simple can't do that…yea you are not going to see the toxin but you could see the mold.

Well I think you can do toxins and pesticides but it traditionally hasn't been very useful because our export customers are interested in lists of hundreds and they don't care about one, so that would be very difficult to deal with;
--except for bacterial, that's a very interesting thing.
Well yes; what are the sensitivities on that?
They use it for ?? water.
How do they keep those bacteria from repeatedly, um those bacteria are so good, they eventually chew up those mycotoxins and pesticides? You've got to use a fresh generation every time.
We've got a couple of minutes before the break. Have we decided who's going to be the spokes person?

There was quite a bit of discussion on the importance of variety identification and technology to achieve this. Other interesting discussions were on bioluminescent bacteria for determining certain defects, ELISA methods for starch, and a good discussion on kernel morphology/geometry determinations.

Reporting Session:

Following the breakout sessions, we reconvened as the plenary group for each breakout groups' report. The material presented to the plenary group is presented here, followed by the transcript of each report.

Report from the protein group: Brad Seabourn reporting:

Short Term/Interim

- SDS
  With more gadgetry, mechanization
  Swelling properties
  Protein/Gluten performance

- Mixograph
  Modifying
  Really just need a mixer and a chart
  How does this relate to the Australian's work on the "Z Arm?"
  Water Absorption
  Gluten strength
  Mix time

- Glutomatic
  Might require modification of a salt
  Gluten Index
  Wet Gluten Measurement

- SRC
  For soft wheat
  Application to hard wheat?

- Mid-IR
  Mixing time stability?
  Applicable to breeders?

- NIR
  Calibrate for U.S. wheats.
  FOSS France
  - Wet gluten
  - W (alveograph W value)
  - Ie
  - Zeleny
    (Protein)

Collaboration

FOSS

Industry
GIPSA
ARS

Canadians

3 year plus/Longer term

- Mid-IR-Raman-Mass Spec
  Gluten Structure
  Rheology

- ELISA
  Water Absorption
  Beta Sheet
    Structure/function relationship
    Partial waxy

- Ultrasound
  2 years – frequency response
  3.5 years – mixing ingredients
  5 years – on-line prototype

  Importance of relating to farinograph, mixograph, and cookie spread
  Affects of structure to rheology

- Chip Based (5 plus years)

- PCR DNA
  Variety characteristics
  Useful with blends/mixes?
  Useful at central labs, not elevators

- Discussion of implications of IP and changes in the market (corn, Brazilian soybeans)

How?

- ## Funding

General
  Mid-IR $5,000-$10,000
  US wheat/ARS
  Better Bean Initiative Example
  Importance of leveraging resources
    Private Sources

NAEGA
NGFA
NAMA
NAWG
GEAPS

NAWG – from this meeting to spearhead some emphasis to get $

Longer Term

Need to increase $
NRI Process
Stress importance of structure/function

Short/Near Term

Structure/Function

Transcript of the report from the protein group: Brad Seabourn reporting:

We started with what do we really need? We started with talking about protein quality and one person vehemently said: no use of the word quality. So we changed that to protein characteristics or performance. And we proceeded to list the tests that we can currently do, the parameters P,L,W that we can derive from the alveograph, mix time from the farino also stability and absorption, wet gluten from the glutomatic, then Jan said that many of the things that we would measure by infrared, that FOSS has done some work in France on French wheat, that possibly there are calibrations that might be useful, so maybe there should be some collaboration between GIPSA, ARS, and FOSS to look at those calibrations and see if they can be applied to US wheat and whether or not it works.

We then went from the list of technologies to how do we develop the most promising approaches to characterizing gluten. And we listed the technologies here as NIR, ultrasound, SDS, mid IR, so beginning with the interim short term stuff, addressing sds, maybe this need to be modified with gadgetry, improved instrumentation, mechanization addressing specifically swelling properties, protein gluten performance.

Regarding the mixograph, possibly modifying this, maybe spring settings using a C? or D? or V? arm, just a simple mix and chart process, that can be rapidly done looking at water absorption, gluten strength mixing time.

The glutomatic: possibly changing the buffer system modifying the salt content and addressing the gluten index as a wet gluten measurement itself and then the solvent retention test look at this specifically for soft wheats and it's application to hard wheats.

Continue up here going to mid-IR spectroscopy, what applications we might get immediately regarding mixing time and stability and it's application to addressing breeder issues,

With NIR we go back to work with FOSS, and what those calibrations can do for US wheat.

Looking at the more long term stuff we come back to a combination of Mid-IR, Raman and mass spec techniques addressing gluten structure and rheology parameters, then using an elisa test to address water absorption, the elisa test for some specific protein conformations related to gluten functionality and also elisa for partial waxy determinations. The ultrasound, we found this very interesting and Martin laid out over 2-5 year timeframe what they are looking to do. At 2 years, addressing the frequency response issues, and at roughly 3.5 years, mixing ingredients and at 5

years an on line prototype. Relating all this back to the farinograph and mixograph, effect on cookie spread, and the effect of structure to rheology.

Also thought the chip based technology is something that should be looked at, taking many of the laboratory processes and trying to shrink those down. On a chip they could be rapidly used in the market place. Also PCR, DNA techniques to determine variety characteristics and also how useful it would be to distinguish blends and mixes.

We then proceeded into discussions of identity preserved changes in the market

Finally we went into the funding thing, this could be a big stumbling block is how we get the resources and funds to do that. I think the overall emphasis, at least for the short term, was try to figure out how we can leverage existing money and get those used to address some of these issues. Long term we're going to have to work on getting increased funds. We've got to get private (non gov't) support such as NAEGA, NAWG, NGSA, and NAMA so spearhead these issues with Congress for the gov't side, and get better coop between private and federal sectors to address these issues. Both in short term and regarding the FGIS project, that want to collect wheat samples and do a NIR calibration, I think it would be worth adding a small amount of money and adding mid-IR scans for those same samples so we'd have that info available. Anything else?

We've mentioned collaborations between FGIS and ARS but we'd be remiss is we didn't include the Canadians in that collaborative effort as well.

Report from the non-protein group: Andrew Ross reporting:

- ***Variety/Class (identification of proteins for "wheats"; USDA-California)***

  "Protein"

  Sample Prep
  Luminex (Automated ELISA)
  HPLC
  Chips (chemical)
  Electrophorosis
  ELISA

  DNA – future research

  Capillary electrophoresis

  DECLARATION

- Water Absorption
  NIR (whole grains)
    Protein
    Hardness

  Mixograph (improved/automated?)
  Farinograph (improved/automated?)

- Kernel Geometry

Imaging

Imaging/SKCS
Sieves
Milling yield
NIR

- Starch Waxy Characterization
  ELISA*
  DNA
  Electrophoresis
  Flour Swelling Volume*

  Raman*

  RVA*
  Particle Size
  Imaging
  Granular size
  Waxy (partial)
  *Exists

- Defects: Mycotoxins, Pesticides (exclude insects- FOSS with imaging acoustics)
  Bioluminescent (quality method – existing)
  ELISA

Imaging

Transcript of the report from the non-protein group: Andrew Ross reporting:

…a number of possibilities for dealing with varietal identification based on identification of proteins or ID of DNA that codes the protein, and so we focused on protein and we looked at idea of automated ELISA systems where we would use currently existing or generate antibodies for a limited number of proteins that allowed us to segregate varieties and here's where the R&D would need to go into to. We thrashed about as to whether we were going to do this at an elevator or a central lab. We even brought in a consultant from the blue group (George Lookhart) looking at high priced things –hp's as George calls them—like hplc—they're all possible, we looked at the idea of sample preparation and separations using chips-- this is all possible, we even identified a laboratory that can help us with the antibody development, which is the ARS lab at Albany, CA. Some is possible in 3 yrs w/ coop of chip maker, and the delivery system company.

The 3 yr horizon was toward the DNA and we didn't go into it any further because being from southern Oregon, I have a very strong bias toward varietal declaration. And that would allow us to use centralized labs as a backup and then we wouldn't have the need for speed that we were trying to impose on those tests. So I jumped up and down and said varietal declaration, it's cheap it's cost effective, and others in the group said it might be coming because of other pressures outside our industry. So any comments?

Barrie: a comment about water basis?? ELISA. I think they're great but unfortunately Luminex is going thru a huge crisis just now—well they need the business---they don't have $$ to put into new things and they have to sell what they have right now.

The other issue we dealt with this whole area was the admixtures of varieties and how we were going to deal with that. For me this is a logistics problem and is not dependent on the technology.

How you deal with that. George has suggested that if you were going to apply mixtures to some of these techniques that what you would get out is a mess and we came to agree with that and that's the point where I jumped on the table and said varietal declaration.

Moving on to water absorption. the most promising technology here was NIR reflection , NIR spectroscopy. The possibility of doing it on the whole grain or the possibility of not using a direct calibration for water absorption but to look at protein and hardness as indicators and ….how we develop this. because I was kind of confused with the outcome of this…

--we decided it might be best to whether we have potential if the miller did the right thing, for higher water absorption or lower water absorption, but we couldn't come out with a real number of what that water absorption would be.

--so there was potential. The potential for that grain lot to have high water absorption compared to that one, is what we were trying to indicate. There was an indication that for water absorption, and I see it up here as well, that the o'graphs—are being improved on to get the small semi automated. One of the issues that kept coming up, was that it would give us one, might only give us water absorption, some indications of dough strength, but they weren't going to give you enough information. Any comments?

I think we also said that technologies like that might be beyond the capability of the elevator operator.

Some of us described the experience of trying to introduce even the simplest of wet chemistry can be a challenge.

Kernel conformity—we spent a bit of time looking at the semantics—it was important to come up with a definition that suited what we were trying to do—so kernel conformity, knowing that most kernels were non conformists- we decided this was irrelevant—(laughs) and we've yet to decide whether that means morphology, shape, geometry and all those other indications of the physical manifestations of the grain, we decided that geometry was what we wanted to look at, morphology being those physical characteristics such as apical hairs, was not what we were interested in.

And seeing that imaging or imaging connected to a single kernel singulator were very promising techniques, also we find out that these come up again and again, so the possibilities of using single thing with multiple measures was encrypted in this. Ken Preston was being a real non conformist here and suggested that if we want to look at kernel conformity or non conformity, that we should just simply use a ?? and I don't think we should ignore or dismiss simple tests that are already in existence that do a job. That we don't get rid of them without thinking carefully whether we are going to add value by going to something else.

Kernel geometry then segues into one of our other tasks which was to look at milling yield. And we thought that combining technology, imaging, protein, hardness, kernel shape and size was going to come up as at least some prima fascia indication of what potential milling yield might be. But what Henry suggested was that what we wanted to know was how much endosperm was inside those grains and let the miller decide if he can get that out or not. Does that cover it? Any ?'s.

Starch: we ended up thinking that in the current market, and I'm not going to deny the important of granule size distribution,, what is important is peak viscosity related to the presence or absence of one or more waxiness genes. So that was where we focused, so we went thru the possibilities, although my memory is a little clouded as to whether this was an elisa test that's available to differentiate the three enzymes for the presence or absence, and I know there's a DNA test I know there's electrophoresis. The possibility of using …the RVA, the swelling volume test, we know they can separate high and low swelling, which seems to be the biggest thing that pulls thru the market. And then we get to the end of it, the 3 enzymes we are interested in, the waxy genes or that are produced by the waxy genes are encrypted in varieties, we already know if it's a partial waxy and which partial waxy it is, or if we don't know we should at the breeder level. And so I don't think we

need to do this at all. And so I think it was the consensus that to measure starch characteristics at the primary receival when you already knew the variety, if we did that, was??? Now if we aren't going to look at variety then we have to think about which of these technologies would be most illustrious. The most developed RVA is a single machine, we can get some multiple values out of it. If an ELISA test exists we could run some sort of Luminex automated with 112, and we get the possibility that we could do a lot of things with ELISA as well.

Insects: the most exciting thing that came out was bioluminescence. Existing method used with a bacteria that glows in the dark and if it encounters a toxin it glows less strongly. And seems to be a qualitative indication that there was a toxin present and you could use that to quarantine that grain parcel. There's an indication that there are key toxins that we could add to the elisa plate. so one of the things that I kept stamping my foot about is that one of our mandates from yesterday was to look at the possibility of multiple answers from a single?? test and we see NIR coming up a lot. We saw imaging as a technique coming up a lot. And elisa as a potential technique coming up a lot, that all have the potential to do more than one of the requests that we want to interrogate the grain in more than one way. And make a ?? point that the more information you get, each piece of information increases the cost effectiveness by that proportion.

The big issue here is developing the antibodies to the right ones—we know what steps need to be taken to do that. We didn't identify which ones were in the 3 year horizon and which ones were right out there. Questions?

Discussion of specifics of bioluminescence which wasn't clear.

We've reconsidered imaging not for physical defects, insects but thinking about this multitasking machine which could potentially illuminate the grains as they pass with another wavelength that might make the walls of mycelia fluoresce, you would take not the mycotoxins but the fungal contamination itself, that would be a first indicator that there might be mycotoxins present. You could then quarantine that grain lot and test for mycotoxins. Of course this is not applicable for pesticides. Imagining this then, you don't have a black box, you have a black building where you send your seeds in, they go thru a singulator, they get photographed , calculated, then you get moisture by conductance then you send a sound pulse thru to measure something else and then you crush them and measure the hardness and then they drop out of there and you measure milling yield and you sieve out the bran and the flour goes in and gets extracted, drops into the elisa plate and you learn everything else.

Mik commented (*laughter*) about heavy metals, like cadmium. Andrew comments to the effect that we need to look at the whole market, and if it's important to our customer then it's important to us. If heavy metals are important they will tell us and we'll have to measure.

A discussion of funding: Frank Flora gave a brief presentation on the ARS National Programs and the funding process for National Programs. Floor discussion followed:

TIM: Each of us, for the various factors, put up what we thought were the best approaches.

--But wait. I am confused and need some time to go back to my lab and think about this--

TIM: Lets talk a couple of minutes about funding:

--In our group, we were trying to very legitimately come up with ways to find funds on a short term basis and then strategize on a longer term basis ??? on what's important, Federal sector, private sector. So any thoughts on funding sources? How can we leverage, how can we collaborate, how can we use $50 here $100 there and put together enough to work with.?

--One of the things we talked about in the blue group is the soybean industry's better bean initiative and what they've done is bring government and industry together to really look at where they see the future of their commodity. Very successful…ARS research focus...We've kicked it around a bit internally but are really not in a position to drive this thing. National Association of Wheat Growers and various other organizations who would be. From the standpoint of government research it seems like a viable approach because others have used it successfully. But it does require direct involvement of industry to take advantage of their technology??? measureable ??? whatever is all ???

--I think you said too, that relative to gluten characteristics, you have to show a relationship between structure and function, is that what you said?

--*More discussion of ARS funding and CSRES. Someone asks Frank to comment again on CSRES:* What I mentioned that CSRES would have the primary funds granting program in USDA. The program that probably would have applied to something like this is not likely to be funded by USDA this year and that was the Initiative for Future Ag In Food Systems??? which would have addressed the problem solving multi disciplinary, multi institutional grants of a million dollars or more. I think that was a tradeoff when the undersecretary went for more money in the NRI program, which is an individual investigator smaller grant program. Long term we can still tap into the NRI program to enhance our knowledge of structure function relationships, which down the road will lead to a better understanding of the technology to ???
That's not going to address GIPSA's 3 year needs, so my suggestion was we really need to look at what we can tap into short term in terms of redirecting proposals(?) and redirecting funds and tapping existing programs and leveraging cooperation and coordination. I think that's going to involve who had interest in what, part of this issue is tapping into these priorities that we've identified over the last 2 days, try to lay out a roadmap and workplan as to where we want to go and who is going to do what to accomplish and look for additional funds. I don't know whether GIPSA has any additional funds to put into. Maybe we can challenge ARS can come up with matching funds. Whether we could or not remains to be seen. But these are various avenues we could explore.

--TIM: So who would be, you have Frank here from ARS representing the research side of it, who would be the individual that would take these thoughts and make people responsible; or have people responsible for taking this to the next level; rather than just leaving here in ??? who is it taking from GIPSA's side?

Any other ideas about funding short term or long term? I know Brad said he would need 5 to 10 thousand dollars to change the technology of NIR and take it to the next level. Any other ideas?

--*Comment from Barrie but couldn't understand.*

--I would second that notion (what Barrie said) because. I think obviously we are talking about technology that will involve development of instrumentation and modification of instrumentation, and so the end user or manufacturer is going to have to be involved in this process early on in order for efficient technology transfer to take place and commercialization. So I think the potential manufacturer of any of this equipment has to be at the bar from the beginning.

--And is there a way to tap in to them? Is there any kind of association of equipment manufacturers? Or some way to get their attention on something?
--Manufacturers are always looking for a way to ???

Pat: There's probably a slice of this pie for places like NDSU, KSU, UN-L, and folks like that and Canadian universities-Martin, you could answer that, --looking for extramural funding and sources of extramural funding-- Barrie, you have probably applied for a grant or two in your career--is there some guidance on a clearinghouse or some sort of information for Win-Rock grants or whoever it might be. Barrie would you like to comment on that?

--Yes. Sure. One of the potential ways of going at this might be through the information offices of the universities and creating a ?? of what we ??? and they are actually pretty good at this. And in addition to exploit the potential of getting suppliers money that way through the university people willing to chip in to support a good cause. I know our college of engineering has been excellent at doing that. So that's a good point-using the services of the university as a broadcast medium ?? ??? Actually I was talking to Jim—he's been bugged by our intellectual property officer to consider some of our Karnal bunt work. So they're doing their job of spreading the word. So I think that's one other avenue of a possibility.

The discussion turned to follow up activity. The group agreed that a follow up meeting should be held in 2004, host sight to be determined. We should all be on the lookout for funding sources and make all members of the group aware. We should also stay in touch.

An observation at the close of the meeting: unless someone had a flight to get to, participants tended to stay in the room and discuss potential research collaborations. It seemed as if there were a few "AHA!" insights on the part of several of the participants.

NEXT STEPS:

GIPSA is taking the following next steps:

Electronic Discussion Room: David Shipman suggested and GIPSA staff is developing an electronic discussion room. It will be a *List-Serve* format. All interested person will be able to read the discussion strings but registration will be required to post comments. We hope to have this running in November. We envision this as a convenient way for researchers and others to share their thoughts and insights on the area of focus and hope that everyone who attended the April, 2003 meeting will participate. Anyone seriously interested in posting is invited to register.

National Science Foundation: Andrew Ross has put together a very good proposal for the NSF. He may be looking for collaborators to add component projects. GIPSA enthusiastically endorses Andrew's efforts.

Expanded participation. We made contact with one of the respected scientists from the Australian Wheat Board during the recent American Association of Cereal Chemists meeting in Portland, OR. AWB expressed interest in joining in our discussion of finding rapid methods of end use quality determination as they share in that quest.

Promising technologies discussed were:
NIR calibrations for gluten strength indicators, especially given the European work.
ELISA techniques for protein quality, puroindolines, waxy, and possibly varietal ID.
Lab on a Chip for various quality factors; also strip tests for quality factors.
Ultrasound in the future—discussed the potential for a test on whole ground meal.
Rapid sedimentation volume-
Mid-IR and Raman spectroscopy to add to the body of knowledge for NIR calibration work.
Add-on technology for the SKCS.

In summary, the meeting was successful in that a list of the most important wheat quality factors was generated. Development of rapid technologies for testing wheat for these important quality factors was discussed and several promising technologies were identified. GIPSA challenged the group to deliver the first of these technologies by May 2006. There was consensus that a test could be delivered by that time. Much of the discussion focused on gluten quality, however starch properties such as 'waxy' for products like noodles were also discussed. Testing approaches not requiring whole grain were discussed, e.g., tests using whole meal such as in SDS test or simple mixing tests. Additionally, despite the fact that the US grain handling and merchandising system does not routinely identify varieties, much discussion was given to that area of endeavor. Working with industry partners is crucial. Sub-samples of the wheat sample set GIPSA has gathered for characterization can be utilized for characterization by technologies outside the scope of the GIPSA-ARS project. Finding sources of funding is critical. A grass roots effort by stakeholders is needed.

N.B. Anyone wishing to add clarification to this report or to add follow on comment is invited to do so by sending email to: Patrick McCluskey@usda.gov.